Vol. XI, Part VI December, 1941

THE INDIAN JOURNAL OF AGRICULTURAL SCIENCE

Issued under the authority
of
The Imperial Council of Agricultural Research

Annual subscription
Rs. 15 or 23s. 6d.

Price per part
Rs. 3 or 5s.

PUBLISHED BY THE MANAGER OF PUBLICATIONS, DELHI.
PRINTED BY THE MANAGER, GOVERNMENT OF INDIA PRESS, NEW DELHI,
1941.

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(December 1941)

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ORIGINAL ARTICLES

COLCHICINE-INDUCED POLYPLOIDY IN CROP PLANTS

I. GRAM (*CICER ARIETINUM* L.)

BY

S. RAMANUJAM

AND

A. B. JOSHI

Imperial Agricultural Research Institute, New Delhi

(Received for publication on 26 May 1941)

(With Plates LIV-LVIII)

POLYPLOIDY has been the subject of intensive research for over a quarter of a century among plant-breeders and geneticists. This was due mainly to the fact that polyploidy was found to be of widespread occurrence in nature—more than 50 per cent of the angiosperm species being polyploids—and also to the fact that polyploid plants turned up fairly frequently in experimental cultures of various workers. The mode of origin of these polyploids and their importance in evolution are now fairly well known. The realization of the far-reaching effects of polyploidy in producing new and superior types of plants to the existing ones naturally led to experiments on the artificial production of polyploids.

The earliest discovery in the direction of obtaining polyploids artificially was made by Winkler in 1916 when he attempted to produce graft hybrids in *Solanum*. This method was subsequently developed by Jorgensen [1928] and later employed for various purposes by Lindstrom and Koos [1931], Sansome [1931] and several others. Greenleaf [1938] obtained polyploids from callus shoots of *Nicotiana* and its species hybrids by employing hetero-auxin to induce callus formation. The range of applicability of this method is still not fully worked out. Of the other methods, that of temperature treatment employed by Randolph [1932] proved the most effective. Working with maize he reported the production of about 5 per cent polyploids in his experiments. This method was later used by Dorsey [1936] and Peto [1936] who, however, reported varying successes. Occasional polyploids were also obtained in various other ways : by X-rays [Ichijima, 1934], by bacteria [Kostoff and Kendall, 1932], and by centrifuging [Kostoff, 1937 ; 1938,1], etc.

By far the easiest and the most successful method for inducing polyploidy was discovered recently by Blakeslee and Avery [1937], and Nebel and Ruttle [1938]. These authors working independently showed that colchicine, an alkaloid occurring in the seed and corm of *Colchicum autumnale*, when applied in weak concentrations in water to growing parts of plants produced polyploids. Since this discovery was announced, experiments were undertaken in all parts of the world to test the efficacy of this alkaloid for producing polyploids and the results obtained have in the main fulfilled expectations. A comprehensive review of work done in this connection is given by Fyfe [1939] and Dermen [1940]. Another chemical that has since been reported to produce

similar effects on plants is acenaphthene [Kostoff, 1938,2 ; Navashin, 1938]. The success obtained with this chemical is not as universal as with colchicine [Nebel, 1938 ; Blakeslee, 1939] although it is reported to have certain advantages over the latter [Levan, 1940].

In India work on the production of polyploids by the use of colchicine was taken up at the Imperial Agricultural Research Institute and a number of crop plants subjected to treatment with the chemical have been under study. A preliminary account of striking results obtained with chilli (*Capsicum annuum* L.) was published by Pal and Ramanujam [1939]. Amin [1940] in Surat and Richharia and Persai [1940] in Nagpur have also reported the production of polyploids in cotton and sesamum respectively by the use of colchicine. In this paper an account of work done and results obtained with colchicine treatment of gram is given.

Materials and methods

Seeds of gram variety I P 25 were soaked in water for 24 hours and then placed on moist filter paper for germination. Just at the stage when the radicles began to emerge, the seeds were immersed in aqueous solutions of colchicine of different concentrations for different periods of time. The concentrations employed were 0·25 per cent, 0·5 per cent and 1·0 per cent and the periods of immersion included ½ hour, 2 hours, 6 hours and 24 hours. Ten seeds were used for each treatment, the total number of treatments being twelve. After treatment, the seeds were washed in distilled water and placed on damp filter paper in Petri dishes for further germination. After about a week, the seedlings were planted out separately in pots filled with sterilized soil. For every treatment, seeds soaked in distilled water for corresponding periods were sown as controls.

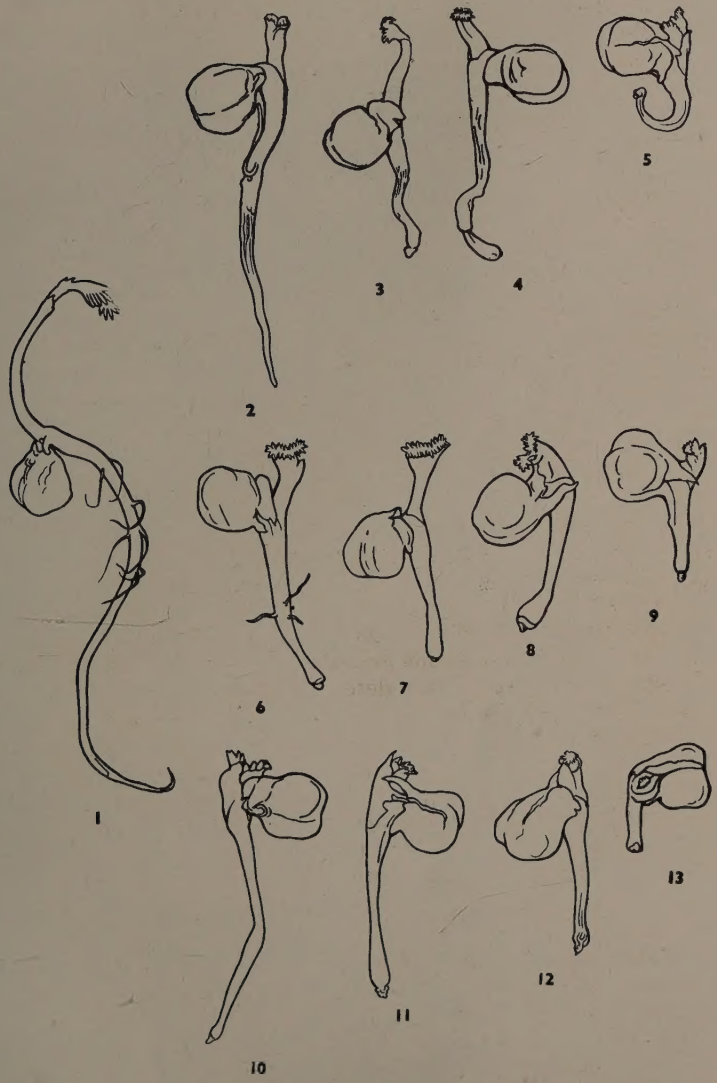
Observations on the growth and flowering of colchicine-treated plants (C_1 generation) and their progenies (C_2 generation) were taken together with controls. The size of stoma guard cells, pollen size and sterility were examined in each case for preliminary detection of induced polyploidy. These observations were supplemented by the study of mitoses in root tips and meioses in pollen mother cells in as many plants as possible. Root tips were fixed in Navashin's fluid and sections stained with iodine-gentian-violet. Meioses in pollen-mother-cells were studied exclusively in acetocarmine smears made permanent by McClintock's schedule [1929]. Drawings were made with the aid of a camera lucida at bench level using a 2 mm. apochromatic objective and 15 × and 20 × compensating oculars.

Morphology of colchicine-treated plants

C_1 generation

The immediate effect of treatment of the seeds was a swelling of the radicles and the plumules and a retardation of their growth compared to the untreated controls. Although all the seeds in any one treatment were not affected to the same extent, the abnormalities induced by treatment appeared to vary with the concentration of colchicine and the duration of treatment. Increased concentrations and longer durations caused a more pronounced swelling and a greater delay in the growth of the plumules and radicles. In some of the heavier treatments the growth of the plumule was completely checked and the radicle began to dry up. Germinating seeds six days after

GERMINATING SEEDS SIX DAYS AFTER TREATMENT



1. Control ; 2–5. Treated with 0·25 per cent colchicine for $\frac{1}{2}$, 2, 6 and 24 hours respectively ; 6–9. Treated with 0·5 per cent colchicine for $\frac{1}{2}$, 2, 6 and 24 hours respectively ; 10–13. Treated with 1 per cent colchicine for $\frac{1}{2}$, 2, 6 and 24 hours respectively

1. Control ; 2–5. Abnormal seedlings due to colenicine treatment

treatment are shown in Plate LIV, figs. 1-13. It will be seen from figs. 5, 9 and 13 that longer durations of treatment produced characteristically stunted and swollen seedlings. Only a very small percentage of such seedlings developed into mature plants. Table I gives the number of plants in the different treatments that grew to maturity and the proportion of polyploids in each treatment.

The total number of plants obtained from treatment with 1·0 per cent solution was considerably less than that from weaker concentrations. Similarly, the total number of plants from longer durations of treatment was less than that from shorter durations. From the data available it would appear that treatment of seeds with 0·25 per cent solution for ½ hour is the best from the points of view of survival of plants and induction of polyploids.

The treated seedlings, after transplantation in pots, continued to grow very slowly compared to the controls ; several of them died in various stages of growth, presumably due to an intolerance to the chemical. Many of the surviving seedlings showed characteristic abnormalities in growth in the early stages, such as curling and twisting of the stem and leaves and a roughening of their surface. The apical bud in some cases stopped growth following a swelling of the apex of the stem and one of the cotyledonary shoots took its place. Plate LV, figs. 1-5 show a few of the abnormal seedlings compared to a control. Later on, the abnormal seedlings grew more or less normally, though slowly, compared to the controls, producing thicker stems, broader and darker leaflets than the latter. Of the 26 plants derived from the treatments, only 13 developed these characteristics of slow growth with thicker stems and darker and broader leaves, the other 13 developing normally like the control plants. The 13 gigas plants came into flower four to five days later than the others. The size of flowers in the gigas plants was bigger than that in the controls. An examination of pollen size and size of stoma guard cells of these plants compared to the controls showed them to be polyploids, the sizes of pollen and stoma guard cells of the polyploids being bigger than those in control plants. Pollen sterility in the polyploid plants varied from 40 to 80 per cent, while that in the control plants did not amount to more than 10 per cent in any case. A cytological examination of meioses in pollen-mother-cells of these 13 plants showed that all of them were tetraploids. In one plant, however, a branch was noticed with considerably broader leaflets and larger stomata, which may have been an octoploid, but this dried up without producing flowers.

C_2 generation

Seeds from individual branches of the 13 tetraploid plants were collected separately and grown in pots during 1939-40 together with controls. The resulting plants were studied carefully with regard to their morphological characters, such as height of plant, number of main branches, number of leaves on the tallest branch, number of leaflets per leaf, length and breadth of leaflets and the standard petal, size of stoma guard cells and pollen size and sterility. The individual plants were also examined for chromosome numbers. The nature of progenies obtained from the different plants tabulated branchwise is given in Table II and the data regarding morphological characters in Table III.

TABLE I

Results of colchicine treatment of gram, I P 25

(1938-39)

Concentration of colchicine ::→	0·25 per cent					0·5 per cent					1·0 per cent					Control		
Duration of treatment ::↓	No. of seeds treated	No. of plants obtained	No. of polyploids	Per cent Survival	Per cent polyploids	No. of seeds treated	No. of plants obtained	No. of polyploids	Per cent survival	Per cent polyploids	No. of seeds treated	No. of plants obtained	No. of polyploids	Per cent survival	Per cent polyploids	No. of seeds	No. of plants obtained	Per cent. survival
1/2 hour . . .	10	10	6	100	60	10	4	1	40	10	10	3	1	30	10	10	10	100
2 hours	10	4	1	40	10	10	2	1	20	10	10	...	...	...	...	10	10	100
6 hours	10	3	3	30	30	10	...	...	...	...	10	...	...	...	...	10	10	100
24 hours . . .	10	...	...	...	...	10	...	...	...	...	10	...	...	...	...	10	10	100

TABLE II

Progenies of colchicine-treated plants, C_2 generation

(Seeds sown branchwise)

(1939-40)

Plant No. in 1938-39	Branch No.	No. of seeds sown	No. of plants obtained	No. of diploids $2n=16$	No. of polyploids $2n=32$	Remarks
2	Did not set seed					
5	5—1	5	2	2	..	
	5—2	1	1	..	1	
	5—3	1	1	..	1	
	5—4	1	1	..	1	
	5—5	2	1	..	1	
	5—6	1	..	..	..	Mixoploid
		11	6	2	4	
7	7—1	1	1	..	1	
	7—2	1	1	..	1	
	7—3	1	1	..	1	
	7—4	2	2	2	..	
	7—5	2	2	..	2	Mixoploid
		7	7	2	5	
8	8—1	5	4	4	..	Mixoploid ?
9	9—1	2	2	..	2	Polyploid
10	10—1	6	6	6	..	
	10—2	8	4	4	..	
	10—3	1	1	..	1	
	10—4	1	1	1	..	
	10—5	3	3	3	..	
	10—6	4	4	4	..	
	10—7	4	4	4	..	
	10—8	1	1	1	..	
	10—9	7	5	5	..	Mixoploid
		35	29	28	1	

TABLE II—*contd*

Plant No. in 1938-39	Branch No.	No. of seeds sown.	No. of plants obtained	No. of diploids $2n=16$	No. of poly-ploids $2n=32$	Remarks
13	13—1	5	5	5	..	
	13—2	1	1	1	..	Mixoploid ?
		6	6	6	..	
23	23—1	1	1	..	1	
	23—2	1	..	..	..	Polyploid
		2	1	..	1	
31	31—1	2	2	..	2	
	31—2	..	..	..	..	
	31—3	2	1	..	1	Polyploid
		4	3	..	3	
44	Did not set seed					
61	61—1	2	1	1	..	
	61—2	1	..	..	..	
	61—3	1	1	..	1	Mixoploid
		4	2	1	1	
62	62—1	9	9	9	..	
	62—2	1	1	..	1	
	62—3	1	1	..	1	Mixoploid
		11	11	9	2	
63	63—1	1	1	..	1	Polyploid

It is seen from Table II that in many cases seeds from different branches have given rise to different types of plants with regard to chromosome numbers. Plants 5, 7, 10, 61 and 62 have given rise to both diploids and tetraploids, thereby showing that the original plants obtained directly from treatment contained mixed $2n$ and $4n$ tissue. Plants 9, 23, 31 and 63 gave rise to only a few seeds, all of which produced tetraploids. In these cases it is likely that the whole plants were of $4n$ tissue. Plants 8 and 13, however, gave rise t only diploids. These plants were originally very sterile and produced only

FIG. 1. Flower and leaf of a diploid plant

FIG. 2. Flower and leaf of a tetraploid plant

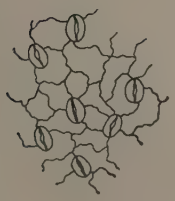


FIG. 3. Stomata on the leaf of a diploid plant

FIG. 4. Stomata on the leaf of a tetraploid plant

FIG. 1. A diploid plant

FIG. 2. A tetraploid plant

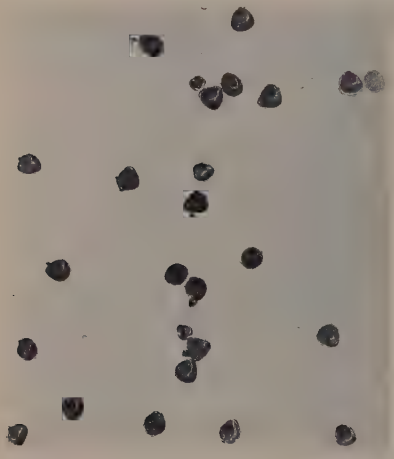


FIG. 3. Pollen from a diploid plant

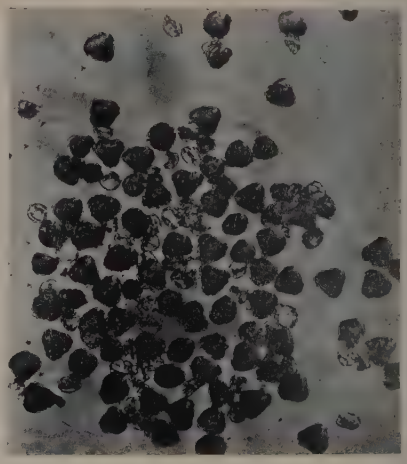


FIG. 4. Pollen from a tetraploid plant

FIG. 5. Pods and seeds of a diploid plant

FIG. 6. Pods and seeds of a tetraploid plant

few branches that set seed. It is probable that in these cases also the plants were mixoploids but that the polyploid branches did not produce seeds to give polyploid progeny. These results clearly show that when seeds are treated with colchicine, mixed 4 *n* and 2 *n* sectors develop on the plants arising from treatment, which causes abnormalities owing to differential growth rates of these tissues and that pure tetraploids are recovered only on growing a further generation.

An examination of Table III brings out some of the morphological changes induced in the gram plants as a result of a quantitative change in chromosome number from a diploid to a tetraploid condition. The cell size in the tetraploid is increased as shown by the stoma guard cells and the pollen. The sizes of the leaflets and the flowers are also increased. The increased size of these plant parts are shown in Plate LVI, figs. 1-4 and Plate LVII, figs. 3 and 4. The pod size and the seed size are also increased in the tetraploid compared to the diploid as shown in Plate LVII, figs. 5 and 6. The height of plants in the tetraploids does not show any significant variation from the diploids although in regard to the number of main branches the diploid has more branches than the tetraploid. These results agree in general with those obtained with other artificially induced polyploids [Kostoff, 1938, 3]. Plate LVII, figs. 1 and 2 are photographs of a diploid and a tetraploid, but the difference in height shown by them is not a general feature as is evidenced by the measurements obtained on a large number of plants. The tetraploid pollen is only partially fertile compared to the diploid, the sterility in the former varying from 40 to 80 per cent, while that in the latter is not more than 10 per cent in any case. Our knowledge of quantitative variations induced by polyploidy and their effects as studied in artificially induced polyploids are as yet meagre as pointed out by Stebbins [1940]. The physiological effects of these quantitative changes on the plants themselves yet remain to be fully investigated and this study was handicapped up to now by the lack of a sure method of obtaining polyploids in large numbers. It is here that the discovery of Blakeslee and others of producing polyploids at will holds great promise for the future.

It must be mentioned that the range of variability of the morphological characters studied is more in tetraploids than in diploids. This may in part be due to secondary effects of the treatment. It is worth while growing the tetraploids for a number of years and in different localities for determination of their reactions to different environments and to natural selection. During the year 1940-41, 62 tetraploid plants were raised and their pollen sterility determined compared to the diploids. The following are the data obtained for the years 1939-40 and 1940-41 :—

Per cent sterility	0-35	36-45	46-55	5665	66-75	76-85
No. of tetraploids (1939-40)	3	7	6	3	2	
No. of tetraploids (1940-41)	25	17	10	4	6	

It would appear from the data that there is a shift in the number of plants towards greater fertility in the C_3 generation. This indication is significant in the light of the observations of Muntsing [1936] that natural selection over a long period of time may lead to increased fertility among auto-polyploids.

TABLE III

Diploids and tetraploids compared with regard to some of the plant characters

(1939-40)

Serial No.	Plant characters	Tetraploids			Diploids			Difference between means (M.D.)	S. E. of difference	M. D. / S. E. of diff.	Remarks
		No. of plants recorded	Mean ± standard error	standard deviation	No. of plants recorded	Mean ± standard error	Standard deviation				
1	Pollen size (mean diameter).	21	43·750μ to 46·875μ		55	34·375μ to 37·500μ					The mean diameter of tetraploids is 1¼ times that of diploids, which is equal to twice the volume.
2	Pollen sterility (range) .	,,	40—80 per cent		,,	Nil to 5 or 10 per cent					Tetra.>Di.
3	Number of main branches .	,,	8·3809 ±0·6813	3·122	,,	10·8± 0·4745	3·5192	2·4191	0·8303	2·91	Di.>Tetra.
4	Number of leaves on the tallest branch	,,	25·85 ±0·3734	1·711	,,	24·54 ±0·2376	1·7619	1·31	0·4426	2·96	Tetra>Di.
5	Height of plant in cm. .	,,	30·26 ±0·7176	3·29	,,	29·49 ±0·4138	3·0686	0·77	0·8284	0·93	Difference not significant
6	Number of leaflets per leaf .	,,	14·03 ±0·1356	0·6221	,,	13·74 ±0·0305	0·2261	0·29	0·1390	2·09	Tetra.>Di.
7	Length of leaflet in cm. .	,,	1·027 ±0·02512	0·1151	,,	0·92 ±0·0105	0·0781	0·1070	0·02723	3·93	Tetra.>Di.
8	Breadth of leaflets in cm. .	,,	0·6452 ±0·01396	0·06403	,,	0·4483 ±0·00453	0·03362	0·1969	0·01468	13·41	Tetra.>Di.
9	Length of the guard cell of stomata in μ	,,	42·68 ±0·2217	1·0159	,,	31·38 ±0·07359	0·5458	11·3	0·2336	48·37	Tetra.>Di.
10	Breadth of the guard cell of stomata in μ	,,	30·97 ±0·1257	0·574	,,	24·21 ±0·09665	0·7168	6·76	0·1586	42·62	Tetra.>Di.
11	Maximum length of flower standard in cm.	19	1·106 ±0·1442	0·0628	30	0·911 ±0·007694	0·04214	0·195	0·01634	11·93	Tetra.>Di.
12	Maximum breadth of flower standard in cm.	,,	0·9194 ±0·01442	0·0628	,,	0·6946 ±0·007176	0·03931	0·2248	0·01611	13·95	Tetra.>Di.
13	Number of days from sowing till flowering	21	65·3	...	55	62·4	...	...	...	...	

Cytology

The chief criteria employed for a preliminary detection of polyploidy in the colchicine-treated plants were based on the increased size of pollen and stoma guard cells. Cytological studies invariably supported the results of these observations.

The chromosome number of *C. arietinum* has been determined by various workers. Dombrowsky-Sludsky [1927], Rao [1929], and Dixit [1932] reported its somatic number as 14. The last-named author, however, reported $2n=16$ as the number in a certain large-seeded Kabuli variety which he called *C. Kabulicum*. The authors working with many varieties studied by Dixit obtained $2n=16$ chromosomes in each case ; in no case was the number $2n=14$ met with. Avdulov [1937], Iyengar [1939], and Richharia and Kalamkar [1938] have also reported the chromosome number of the species as $2n=16$. Polyploid species of *Cicer* are unknown, nor have naturally occurring or artificially produced polyploids of *C. arietinum* been reported.

In the present investigation both mitosis and meiosis of the diploid and the artificially produced tetraploid plants of I P 25 were studied. In the root tips of the diploid, 16 chromosomes (Plate LVIII, fig. 1) were clearly counted in several cells while in the tetraploids 32 chromosomes (Plate LVIII, fig. 2) were clearly seen. In pollen meiosis in the diploid, eight bivalents were found at diakinesis and mataphase I (Plate LVIII, fig. 3) and these underwent regular separation at anaphase I (Plate LVIII, fig. 4). At metaphase II, 8 chromosomes in each nucleus were noticed (Plate LVIII, fig. 5). The second division was also regular leading to the formation of normal tetrads. Pollen sterility in the diploids varied from 0 to 10 per cent. In the tetraploids varying numbers of quadrivalents and bivalents were formed at diakinesis and metaphase I, the total number of chromosomes in each cell being 32. An examination of 34 cells at diakinesis and metaphase I in which the chromosomes were clearly spaced out gave the following distribution of quadrivalents :—

No. of quadrivalents	0	1	2	3	4	5	6	7	8	
Number of cells	0	0	0	2	1	8	7	12	4	= 34

The maximum number of quadrivalents was noticed in 4 out of 34 cases, while seven quadrivalents per cell appeared to be of frequent occurrence. Plate LVIII, figs. 6-8 show different numbers and shapes of quadrivalents occurring in three cells. Anaphase I was fairly regular in many cells and the chromosomes were distributed equally, i.e. 16 and 16 to the two poles. The following gives the frequencies of distribution of chromosomes to the two poles at anaphase I.

Distribution of chromosomes	16+16	17+15	18+14
Number of cells	16	6	2

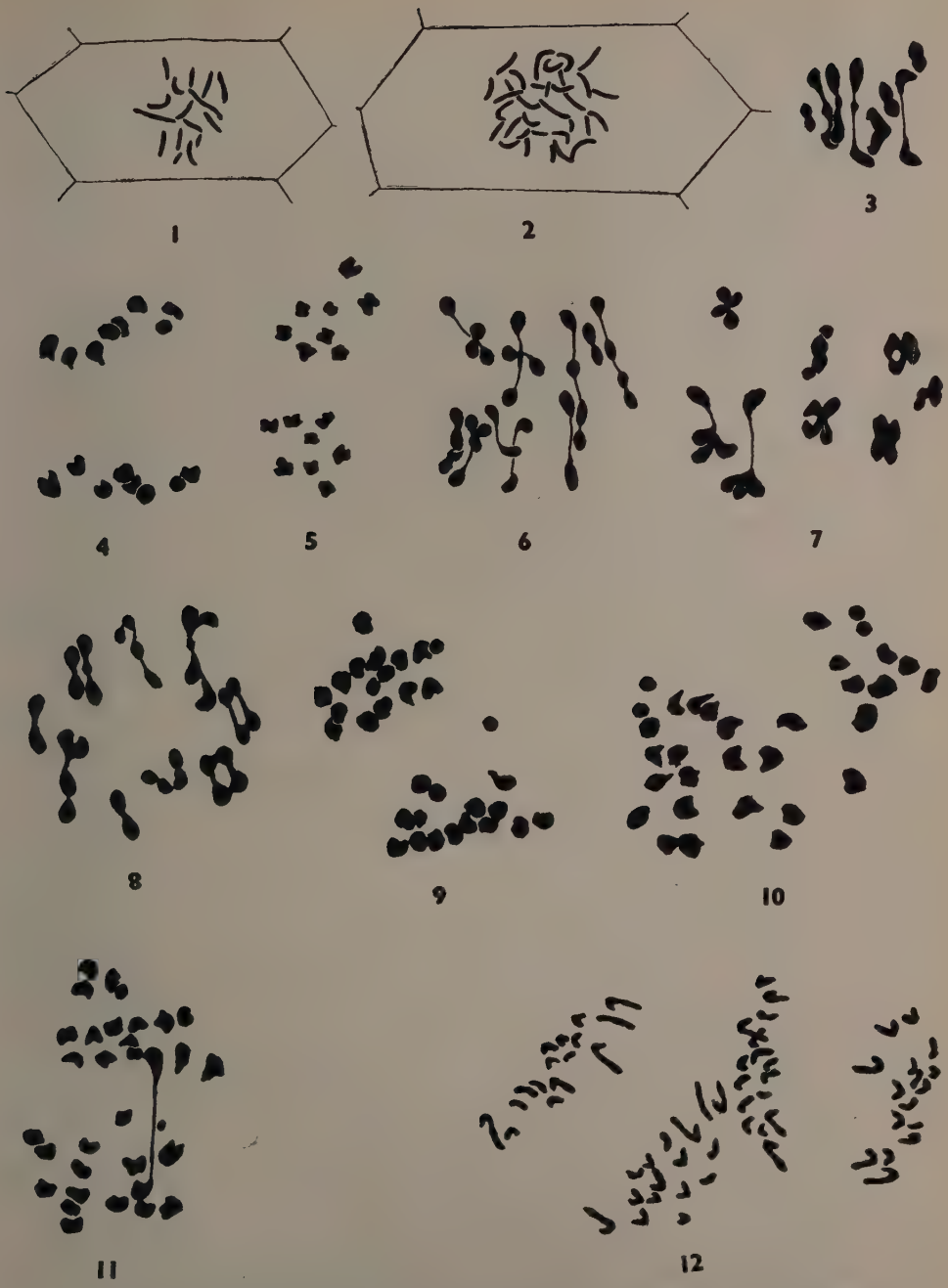
Plate LVIII, figs. 9 and 10 represent cells with equal and unequal distribution of chromosomes to the poles at anaphase I. Occasionally, in some cells one chromosome was seen to lag at anaphase I and get excluded from the daughter nuclei or was seen to divide into half chromosomes and move to opposite poles. In one plant with about 80 per cent pollen sterility, a chromatin bridge with a fragment (Plate LVIII, fig. 11) was noticed at anaphase I in a few cells. This obviously has resulted from the occurrence of an inverted segment in one of

the chromosomes. No such inversion bridges were noticed in the diploid material examined. The second division in the pollen-mother-cells of tetraploids was also fairly regular with 16 chromosomes going to each of the four poles (Plate LVIII, fig. 12). Irregularities at division II, such as the presence of straying chromosomes, etc. were rather rare. In spite of the fairly regular divisions the sterility of pollen in the different tetraploids varied from 40 to 80 per cent. It is noteworthy that progenies of tetraploid plants consisted of only tetraploids ; this is presumably owing to the functioning of only $2n$ gametes to the exclusion of others.

Discussion

It is now well known that the effect of colchicine in doubling the chromosome number of flowering plants is of rather general application, although different species or even related species may require different treatments for successful results. This knowledge has opened out new opportunities to the breeder who is interested in the production of new and improved types of economic plants. The doubling of chromosomes can be induced in fertile species or in sterile species hybrids ; in the former case partially sterile auto-polyploids are obtained and in the latter more or less fertile allo-polyploids are produced. Allo-polyploids are known to have played an important part in evolution as has been demonstrated not only by the numerous and well-known instances of artificial species, such as *Primula kewensis* [Newton and Pellow, 1929], *Nicotiana digluta* [Clausen and Goodspeed, 1925] *Aegilotricum* [Katayama, 1935] *Raphanobrassica* [Karpechenko, 1927] and others but also by the experimental synthesis of natural species like *Galeopsis Tetrahit* [Muntzing, 1932]. Further evidence of their importance in evolution is provided by those of our cultivated plants whose polyploid origin has been worked out, as in the case of wheat [Aase, 1930 ; 1935], cotton [Skovsted, 1934], tobacco [Kostoff. 1936], etc. The importance of allo-polyploidy in evolution lies in the fact that it induces far-reaching changes in sterile species hybrids ; allo-polyploidy is known to have changed a self-sterile species into a self-fertile form, a dioecious into a hermaphrodite race and an annual into a perennial. It is also known to have induced resistance to diseases, pests, frost and drought conditions. As a result of these distinctive features and an increased rate of mutation in them, allo-polyploids are better suited for the production of altogether new forms. While the study of species hybrids, therefore, occupied an important place in the programme of the breeder, its scope was limited by the lack of a sure method of doubling the chromosome number. The discovery of this new method has given an impetus to this line of work and already several artificial polyploids by the use of colchicine have been obtained. Fertile amphidiploids from sterile species hybrids in *Nicotiana* [Warmke and Blakeslee, 1939 ; Kostoff, 1938,4 ; Smith, 1939], in cotton [Beasley, 1940 ; Harland, 1940 ; Kasparayan, 1940], and in wheat [Zhebrak, 1939,1 ; 1939,2], have been obtained which promise results of economic value. Further studies in this direction are full of possibilities for the future of plant breeding.

Auto-polyploidy, on the other hand, has given rise to a great diversity of opinion with regard to its role in evolution. Auto-polyploids usually do not show differences in morphological character from the diploids except for certain size variations induced by a quantitative change in their chromosome



1-2. Somatic chromosomes in the root tips of a diploid and a tetraploid gram respectively ; 3-5. Pollen meiosis in the diploid showing metaphase I, anaphase I, and metaphase II respectively ; 6-8. Pollen meiosis in the tetraploid showing different configurations of quadrivalents ; 9-10. Pollen meiosis in the tetraploid showing late anaphase I with equal and unequal distributions, respectively, of chromosomes to the poles ; 11. Pollen meiosis in the tetraploid showing anaphase I with a chromatin bridge and a fragment ; 12. Pollen meiosis in the tetraploid showing anaphase II

number. They, however, exhibit certain physiological differences such as the slower rate of development, later time of blooming, and an inability to cross with the diploids which give them in many cases a different geographical or ecological distribution. How far are these changes induced by auto-polyploidy effective in evolution ? The question is discussed at length by Muntzing [1936] who by an analysis of intra- and interspecific chromosome forms and experimental auto-polyploids adduces arguments in favour of auto-polyploidy being a factor in evolution. Although Jorgensen [1928], Blakeslee [1921], and Babcock [1934] agree with Muntzing in considering that auto-polyploidy has played a part in evolution, other workers like Afzelius [1924], Clausen [1926], and Navashin [1927] think that new polyploid species cannot possibly arise only ' by multiplication of the same genome ', i.e. auto-polyploidy. Stebbins [1940] discussing the same question states. ' Furthermore, due to the fact that polyploid species are more infertile than their diploid prototypes, an auto-tetraploid is unlikely to maintain its purity unless it is completely isolated not only from its diploid progenitor, but from its polyploid relations as well. Would such a completely isolated race, which would have to become highly inbred, and in which the visible mutation rate is greatly reduced, be likely to give rise to a new line of evolution ? I doubt it.' Muntzing [1936] considering the question of difference in fertility between polyploid chromosome races and experimental auto-polyploids—the former are more fertile than the latter—states that ' this difference is explicable by the fact that chromosome races in contradistinction to the experimental polyploids have been subject to natural selection. They (the polyploid chromosome races) represent the successful survivors from a large material. It is also possible that secondary processes of various kinds have caused an increase in fertility.' It is now recognized that side by side with all conscious artificial breeding there is at work, more or less actively, a certain natural selection. It has been shown that from a very varied population of types, such as that represented by an F_2 generation of an interracial cross, natural selection eliminates a number of types with astounding rapidity. In such populations within about ten years often up to about 75 per cent of all the types originally present die out, although complete uniformity of the surviving type is not attained. It is also clear that if the same initial population is cultivated as a parallel test under different conditions of soil and climate, selection in the different places acts in different directions ; in each place a different mixture of lines survives. Experimental evidence as to how artificially produced auto-polyploids react to their normal and changed environments is rather meagre. Thanks to the discovery of Blakeslee and others who have given us an easy method of producing artificial auto-polyploids in large numbers, this line of study is receiving greater attention. If auto-polyploids, which usually show a large range of variability in the induced characters, are grown over a long period of time and in different localities subjecting them to natural and artificial selection, sufficient data would be obtained with regard to evolutionary trends.

To test the utility of these gram tetraploids, they will now be grown in large numbers in different tracts for a number of years in bulk and the final types will be studied and subjected to artificial selection later on. The same will be done with artificial polyploids of other crop plants produced in the

Institute and the data that will be obtained are expected to give interesting results. It is. of course. understood that natural selection does not always act in the directions desired by the breeder, but conscious selection in the later stages may help to isolate the desired types. In this connection another possible use of auto-polyploids may be mentioned and that is the formation of new allo-polyploids by hybridization of different auto-polyploids, as for example, *Iris Syndetica* [Simonet, 1935].

Another possibility of the utilization of this discovery for breeding in the case of inter-racial crosses is also indicated by Blakeslee [1939]. In nearly every case of inter-racial crosses, which produces fertile progenies, a large number of mendelian factors are at work, so that in the F_2 generation the number of combinations to be expected is very great. Excepting in the case of vegetatively propagated plants, the task of finding in the F_2 or later generations an individual with desired characters in a homozygous condition is full of difficulties. A large number of plants will have to be sown and for many generations to get the desired result. In such a case, Blakeslee thinks that if we could find out a method whereby haploids could be produced by parthenogenesis, the task of getting homozygous diploids in the F_2 generation is made easy by the application of this method. Starting with a highly heterozygous plant, such as a fertile species hybrid, haploids produced from it by parthenogenesis of reduced eggs will contain only one kind of each chromosome, and now if the haploids are doubled by colchicine, homozygous seeds with each chromosome duplicated will result. The plants from these seeds will be homozygous containing different combinations of the genes of the parents, and selection among them will give the desired results in two jumps. The importance of obtaining homozygous diploids for breeding was also stressed by R. C. C. [1936] when he states that ' instead of growing acres of seedlings and throwing all but a handful away, there may come a time when our future Burbanks will grow acres of haploids to get a few dozen seeds—each one of them representing the beginning of a pure line—which will form the basis of later breeding experiments.' Now that we have a means of readily doubling the chromosome number of the haploids, the great need in our genetic programme is to discover a method whereby the chromosome number in a plant could be halved at will.

Summary

Germinating seeds of gram I P 25 were subjected to varying treatments with colchicine and the C_1 and C_2 plants studied with reference to induced polyploidy. Treatment of seeds with 0·25 per cent aqueous solution of colchicine for half an hour gave the best result from the points of view of survival of seedlings and induction of polyploidy. In the generation immediately following the treatments, the affected plants showed a mixture of $2n$ and $4n$ tissue and in the subsequent generation pure tetraploids were obtained.

A quantitative study of the morphology of the diploid and tetraploid plants was undertaken and the results showed that the tetraploids possessed a larger number of leaves per plant, bigger leaflets, bigger flowers, bigger pods and seeds than the diploids. The pollen grains and the stoma guard cells of the tetraploid were also bigger than those of the diploids, although the pollen

grains of the former were about 40-80 per cent sterile compared to only 0-10 per cent sterility of the latter. With regard to height of plants the tetraploids did not vary significantly from the diploids although the latter had significantly more branches than the former.

The chromosome numbers of the tetraploids and diploids were determined as 32 and 16 respectively. The meiosis in the tetraploids was fairly regular with the formation of varying numbers of quadrivalents at diakinesis and metaphase I, seven quadrivalents per cell occurring most frequently. One tetraploid plant, however, showed a chromatin bridge and a fragment in a few cells at anaphase I. The tetraploid plants gave rise invariably to tetraploid progenies.

The significance of the use of colchicine for practical breeding is discussed.

We are thankful to Dr B. P. Pal, Imperial Economic Botanist, for kindly reading through the manuscript and making some useful suggestions.

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STUDIES IN THE TECHNIQUE OF FIELD EXPERIMENTS

V. SIZE AND SHAPE OF BLOCKS AND ARRANGEMENT OF PLOTS IN COTTON TRIALS

BY

V. G. PANSE

Institute of Plant Industry, Indore, Central India

(Received for publication on 12 May 1941)

(With two text-figures)

THE application of statistical methods to agricultural research has led to two important developments. On the agronomic side, complex experiments enabling the experimenter to test the simultaneous effect of two or more treatments have become common, while with the growing appreciation that statistical methods are as necessary and appropriate in plant breeding as in agronomic research, breeders have started to undertake properly laid-out varietal trials containing an increasing number of varieties. To maintain an adequate level of accuracy in experimental comparisons, however, the size of block and consequently the number of treatments or varieties to be included in it cannot be increased beyond a limit. To keep the block size small, experimental designs have been evolved in which by adopting ' confounding ' it is possible to include in one block only a part of the total number of treatments or varieties to be tested [Fisher, 1936 ; Yates, 1936, 1, 2].

The gain in accuracy due to smaller blocks resulting from confounding is offset by certain disadvantageous features. In confounding treatments, comparisons of high order interactions which are considered relatively unimportant are either partially or completely lost. With varieties all comparisons are of equal interest and the theoretical efficiency of confounded arrangements is lower than of an ordinary randomized block lay-out ; but this is expected to be more than counterbalanced by a reduction in the actual error variance. Moreover, all varieties in a trial cannot, in some confounded designs, be compared with the same level of accuracy. With the complexity of design, greater care in field work is necessary and the arithmetical computations in the statistical analysis of the results become rather intricate. For these reasons, an experimenter would not wish to sacrifice the simplicity and flexibility of ordinary randomized blocks by adopting confounding, unless there is a possibility that the desired standard of accuracy cannot be attained by employing a simple lay-out. A knowledge of the change of efficiency with increasing size of block in simple randomized block trials is, therefore, of considerable practical interest in setting an upper limit to the size of block and hence to the number of treatments or varieties which can be tested without resorting to complex experimental design.

While discussing the efficiency of different designs, Yates [1935] has pointed out the need of investigating the increase in experimental error due to increased size of block. With the rapid development of experimental work in cotton conducted at the Institute, this need was increasingly felt and an investigation was carried out with the help of data from a uniformity trial described by Hutchinson and Panse [1935]. The results are presented in this paper. The relative accuracy of alternative designs based on confounding is also discussed. While the conclusions are subject to the usual limitations attached to the results of a single experiment, experience has shown that the previously published results [Hutchinson and Panse, 1935] from this uniformity trial have provided a useful guidance for conducting cotton trials in various parts of Central India and Rajputana, and it is hoped that the present conclusions will similarly be applicable over a wide range of conditions.

Method of analysis

The uniformity trial, it will be remembered, consisted of 1,280 units square in shape and 1/2,000 acre in area. There were 40 units along the length of cotton rows and 32 units across the rows. The yields of unit plots are given in the appendix.

For the purpose of the present investigation, plots of four sizes, 1/50, 1/100, 1/200 and 1/500 acre, were considered. The first two sizes are normally used in agricultural trials, while the last two represent small plots which are necessary under certain conditions, as, for example, when the amount of seed or land is limited. Subject to the restriction that the whole area of the uniformity trial was utilized in every combination, plot shape was also varied and both long and narrow plots as well as more compact ones were examined. Plots of each size and shape were grouped together in blocks of 2, 4, 8, 16 or more plots, 160 being the maximum number of plots per block when plot size was 1/500 acre. Plots were arranged in one or more rows to form a block. In all, 108 combinations were analysed.

The advantage of using blocks in reducing experimental error by removing a larger proportion of variability is expressed as block efficiency. This is a ratio of the error variance that would have been obtained if there were no blocks to that actually obtained after eliminating differences due to blocks. The advantage due to blocks must be considered to break down when this ratio is in the neighbourhood of unity. As a direct measure of accuracy of any experimental arrangement in blocks, the standard error per cent per plot was also calculated. The results are shown in Table I. To save space arrangements of plots in more than four rows have been omitted as these present no new feature.

Block efficiency

The importance of block size, block shape and arrangement of plots in determining efficiency was indicated in the previous analysis of this uniformity trial [Hutchinson and Panse, 1935]. Table I furnishes more extensive material for studying these points.

TABLE I

Block efficiency and standard error per cent for plots and blocks of different sizes and shapes

Size of block		Plots in one row			Plots in two rows			Plots in four rows		
No. of plots	Area (acre)	Block shape*	Efficiency	S. E. (per cent)	Block shape	Efficiency	S. E. (per cent)	Block shape	Efficiency	S. E. (per cent)
				(a) Plot size 1/500 acre, plot shape 4 : 1*						
2	1/250	2 : 1	2·45	14·32						
4	1/125	1 : 1	2·41	14·42	4 : 1	1·70	17·17			
8	1/62·5	1 : 2	2·06	15·61	2 : 1	1·70	17·16			
16	1/31·25	1 : 4	1·95	16·02	1 : 1	1·57	17·87			
32	1/15·62	1 : 8	1·49	18·36	1 : 2	1·54	18·05			
64	1/7·81				1 : 4	1·35	19·28			
				(b) Plot size 1/500 acre, plot shape 1 : 1						
2	1/250	1 : 2	3·01	13·14	2 : 1	2·16	15·53			
4	1/125	1 : 4	2·22	15·30	1 : 1	2·24	15·24	4 : 1	1·63	17·86
8	1/62·5	1 : 8	2·12	15·66	1 : 2	1·97	16·26	2 : 1	1·65	17·75
16	1/31·25	1 : 16	1·47	18·79	1 : 4	1·88	16·62	1 : 1	1·53	18·40
32	1/15·62				1 : 8	1·46	18·86	1 : 2	1·51	18·55
64	1/7·81							1 : 4	1·33	19·75
				(c) Plot size 1/200 acre, plot shape 10 : 1						
2	1/100	5 : 1	4·03	9·38						
4	1/50	2·5 : 1	3·57	9·97	10 : 1	1·09	18·02			
8	1/25	1·25 : 1	2·72	11·41	5 : 1	1·17	17·44	20 : 1	0·96	19·19
16	1/12·5	1 : 1·6	2·48	11·96	2·5 : 1	1·14	17·66	10 : 1	0·99	18·94
32	1/6·25	1 : 3·2	1·72	14·34	1·25 : 1	1·14	17·65	5 : 1	0·99	18·88
64	1/3·12				1 : 1·6	1·13	17·75	2·5 : 1	1·01	18·74
				(d) Plot size 1/200 acre, plot shape 2·5 : 1						
2	1/100	1·25 : 1	4·00	9·51						
4	1/50	1 : 1·6	2·63	11·73	2·5 : 1	3·33	10·42			
8	1/25	1 : 3·2	2·47	12·09	1·25 : 1	2·62	11·75	5 : 1	1·26	16·92
16	1/12·5	1 : 6·4	1·67	14·70	1 : 1·6	2·40	12·26	2·5 : 1	1·13	17·86
32	1/6·25				1 : 3·2	1·70	14·59	1·25 : 1	1·13	17·85
64	1/3·12							1 : 1·6	1·12	17·94
				(e) Plot size 1/100 acre, plot shape 20 : 1						
2	1/50	10 : 1	2·33	7·78						
4	1/25	5 : 1	2·00	8·39	20 : 1	1·21	10·79			
8	1/12·5	2·5 : 1	1·56	9·50	10 : 1	1·00	11·85			
16	1/6·25	1·25 : 1	1·49	9·71	5 : 1	1·00	11·87			
32	1/3·12	1 : 1·6	1·41	9·99	2·5 : 1	1·03	11·69			
				(f) Plot size 1/100 acre, plot shape 5 : 1						
2	1/50	2·5 : 1	5·11	7·82						
4	1/25	1·25 : 1	3·36	9·66	5 : 1	1·06	17·21			
8	1/12·5	1 : 1·6	3·04	10·14	2·5 : 1	1·10	16·85			
16	1/6·25	1 : 3·2	1·89	12·87	1·25 : 1	1·14	16·58			
32	1/3·12				1 : 1·6	1·14	16·57			
				(g) Plot size 1/100 acre, plot shape 1·25 : 1						
2	1/50	1 : 1·6	2·75	10·76						
4	1/25	1 : 3·2	2·89	10·50	1·25 : 1	3·16	10·04			
8	1/12·5	1 : 6·4	1·77	13·40	1 : 1·6	2·91	10·46	2·5 : 1	1·10	17·03
16	1/6·25				1 : 3·2	1·86	13·10	1·25 : 1	1·13	16·76
32	1/3·12							1 : 1·6	1·14	16·74
				(h) Plot size 1/50 acre, plot shape 40 : 1						
2	1/25	20 : 1	1·41	5·86						
4	1/12·5	10 : 1	1·17	6·42						
8	1/6·25	5 : 1	1·05	6·77						
16	1/3·12	2·5 : 1	1·12	6·56						
				(i) Plot size 1/50 acre, plot shape 10 : 1						
2	1/25	5 : 1	2·48	6·72						
4	1/12·5	2·5 : 1	1·72	8·07	10 : 1	0·91	11·11			
8	1/6·25	1·25 : 1	1·68	8·15	5 : 1	0·96	10·79			
16	1/3·12	1 : 1·6	1·58	8·42	2·5 : 1	1·03	10·43			
				(j) Plot size 1/50 acre, plot shape 2·5 : 1						
2	1/25	1·25 : 1	39·64	8·87						
4	1/12·5	1 : 1·6	3·61	8·91	2·5 : 1	0·99	17·04			
8	1/6·25	1 : 3·2	2·01	11·93	1·25 : 1	1·10	16·11	5 : 1	0·92	17·59
16	1/3·12				1 : 1·6	1·14	15·85	2·5 : 1	0·99	17·00

*Throughout this paper shape of plots and of blocks is expressed as a ratio of length to breadth. The side along the cotton rows is treated as length and that across the rows as breadth irrespective of magnitude.

An inspection of the table shows that for plots with a given size, shape and arrangement, efficiency gradually decreases with increasing size of block. The largest blocks (1/3·12 acre) have a very poor efficiency everywhere, except when compact blocks of this size are formed by arranging 1/100 or 1/50 acre plots in one row. Blocks of identical size and shape formed by arranging longer plots in one or two rows are more efficient than those with shorter plots of the same size arranged in two or more rows. While the difference is usually small, the advantage in favour of long plots arranged in one row is strikingly shown when blocks of 1/100 acre plots with a shape of 20 : 1 or of 1/50 acre plots with a shape of 10 : 1 are compared with blocks of shorter plots of the same sizes. A reason for this difference is that while variation between blocks of a given size and shape is constant, the total variability of long and narrow plots is less than that of shorter plots of the same size, particularly where the plot size is large.

The influence of block shape on efficiency is observed when blocks of the same size but of different shapes formed with plots of a given size arranged in a given number of rows are compared. The general conclusion is that the more compact blocks have a higher efficiency. Only in 10 out of about 60 comparisons is an opposite tendency observed. In four of these exceptional cases, less compact blocks formed with longer plots show a higher efficiency than more compact blocks with shorter plots. Here the reduction in total variability due to longer plots appears to have outweighed the increased variability between more compact blocks. In three other cases where plots are arranged in two rows, less compact blocks with shorter plots possess a higher efficiency than more compact blocks with longer plots. Probably a new factor is responsible for this discrepancy. Plots arranged in such a way that their ends or shorter sides are in contact will be less strongly correlated than when their longer sides form the common border and will have a greater variability. Variability from this cause will increase as the length of plot increases in proportion to its breadth, leading to a loss of efficiency when long and narrow plots are arranged in two or more rows.

The effect of block shape and of arrangement of plots on efficiency when plots of a given size and shape are selected for experimentation is a point of more direct practical interest. It will be seen from Table I that in the majority of cases arrangement of plots in one row has proved more compact and also more efficient than in two or more rows. Those cases in which arrangement in one or two rows is less compact than in two or four rows respectively are brought together in Table II.

When by arranging plots in two rows instead of one, the length-breadth ratio of blocks is considerably reduced, as from 1 : 16 to 1 : 4 or from 1 : 6·4 to 1 : 1·6, the former arrangement is distinctly more efficient. With short plots of 1/200 or 1/100 acre size, a comparatively smaller change in block shape in the right direction, for example, from 1 : 3·2 to 1·25 : 1, also improves efficiency slightly ; but a similar change brought about by arranging longer plots in two rows is disadvantageous. Even with short plots an arrangement in four rows is less efficient than in two rows for small increases in the compactness of blocks. It would thus appear that unless blocks were made substantially more compact by arranging long plots in two rows or short plots in four or more rows, an arrangement in one row for long plots and in one or two rows for short plots is to be preferred.

TABLE II

Block shape and efficiency when arrangement in two or more rows is more compact

Size of block		Plots in one row		Plots in two rows		Plots in four rows	
No. of plots	Area (acre)	Block shape	Efficiency	Block shape	Efficiency	Block shape	Efficiency
		(a) Plot size 1/500 acre, plot shape 4 : 1					
16	1/31·5	1 : 4	1·95	1 : 1	1·57		
32	1/15·62	1 : 8	1·49	1 : 2	1·54		
		(b) Plot size 1/500 acre, plot shape 1 : 1					
4	1/125	1 : 4	2·22	1 : 1	2·24		
8	1/62·5	1 : 8	2·12	1 : 2	1·97		
16	1/31·25	1 : 16	1·47	1 : 4	1·88	1 : 1	1·53
32	1/15·62			1 : 8	1·46	1 : 2	1·51
		(c) Plot size 1/200 acre, plot shape 10 : 1					
32	1/6·25	1 : 3·2	1·72	1·25 : 1	1·14		
		(d) Plot size 1/200 acre, plot shape 2·5 : 1					
8	1/25	1 : 3·2	2·47	1·25 : 1	2·62		
16	1/12·5	1 : 6·4	1·67	1 : 1·6	2·40		
32	1/6·25			1 : 3·2	1·70	1·25 : 1	1·13
		(e) Plot size 1/100 acre, plot shape 5 : 1					
16	1/6·25	1 : 3·2	1·89	1·25 : 1	1·14		
		(f) Plot size 1/100 acre, plot shape 1·25 : 1					
4	1/25	1 : 3·2	2·89	1·25 : 1	3·16		
8	1/12·5	1 : 6·4	1·77	1 : 1·6	2·91		
16	1/6·25			1 : 3·2	1·86	1·25 : 1	1·13
		(g) Plot size 1/50 acre, plot shape 2·5 : 1					
8	1/6·25	1 : 3·2	2·01	1·25 : 1	1·10		

There is one combination with 1/200 acre plots in which a more compact block (shape 1 : 1·6) in one row has proved less efficient than an arrangement in two rows with block shape 2·5 : 1. No explanation can be offered for this result.

STANDARD ERRORS

Standard error per plot is the measure of efficiency of an experimental lay-out. Table I shows that for plots of the same size and shape, the standard

error per cent steadily decreases as block efficiency increases ; but comparing plots of different sizes and shapes it is seen that lay-outs with large plots and with long and narrow plots have lower standard errors irrespective of block efficiency. The relation between standard error per cent and block efficiency is well brought out by plotting them on graph paper (Fig. 1).

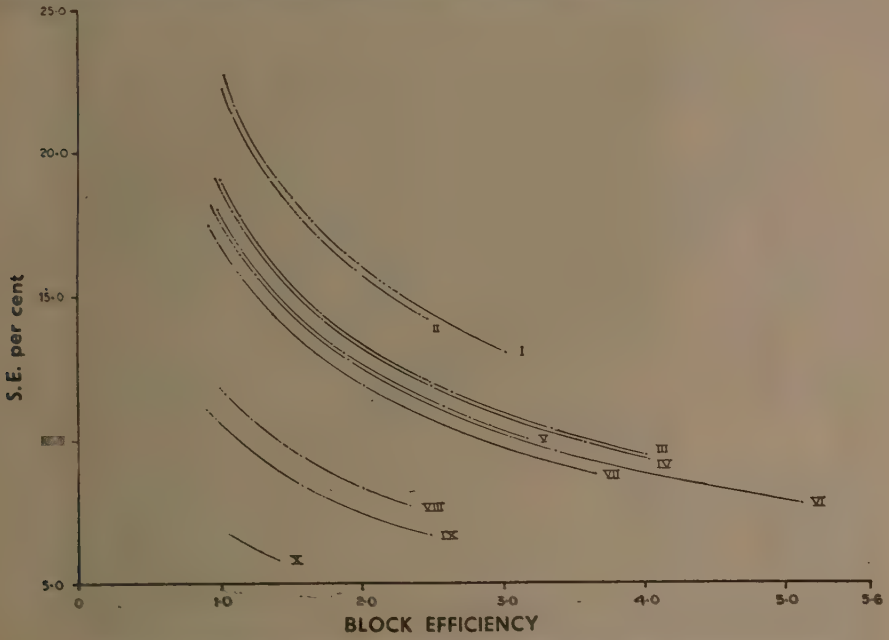

FIG. 1. Relation between standard error per cent and block efficiency

Curve	Plot size (acre)	Plot shape (length : breadth)	Curve	Plot size (acre)	Plot shape (length : breadth)
I	1/500	1 : 1	VI	1/100	5 : 1
II	1/500	4 : 1	VII	1/50	2·5 : 1
III	1/200	2·5 : 1	VIII	1/100	20 : 1
IV	1/200	10 : 1	IX	1/50	10 : 1
V	1/100	1·25 : 1	X	1/50	40 : 1

The points lie on smooth curves, a separate one for plots of each size and shape. The predominant influence of plot size and shape on the standard error of a lay-out is now obvious. Plots of 1/50 acre size generally have the lowest standard error ; but plots of 1/100 acre provided they are sufficiently long and narrow (shape 20 : 1), have a considerably lower error than 1/50 acre plots which are short and broad (shape 2·5 : 1). With the two smaller plot sizes, shape is unimportant, but even here long and narrow plots have a slightly reduced error. With blocks of the same efficiency, experiments with larger and longer plots will have a lower standard error. The efficiency of a lay-out is thus primarily determined by plot size and shape, and after these have been selected, an arrangement giving the maximum efficiency of blocks should be aimed at.

Curves in Fig. 1 represent logarithmic functions and are reduced to straight lines when logarithms of standard error per cent are plotted against those of block efficiency. They are shown in Fig. 2.

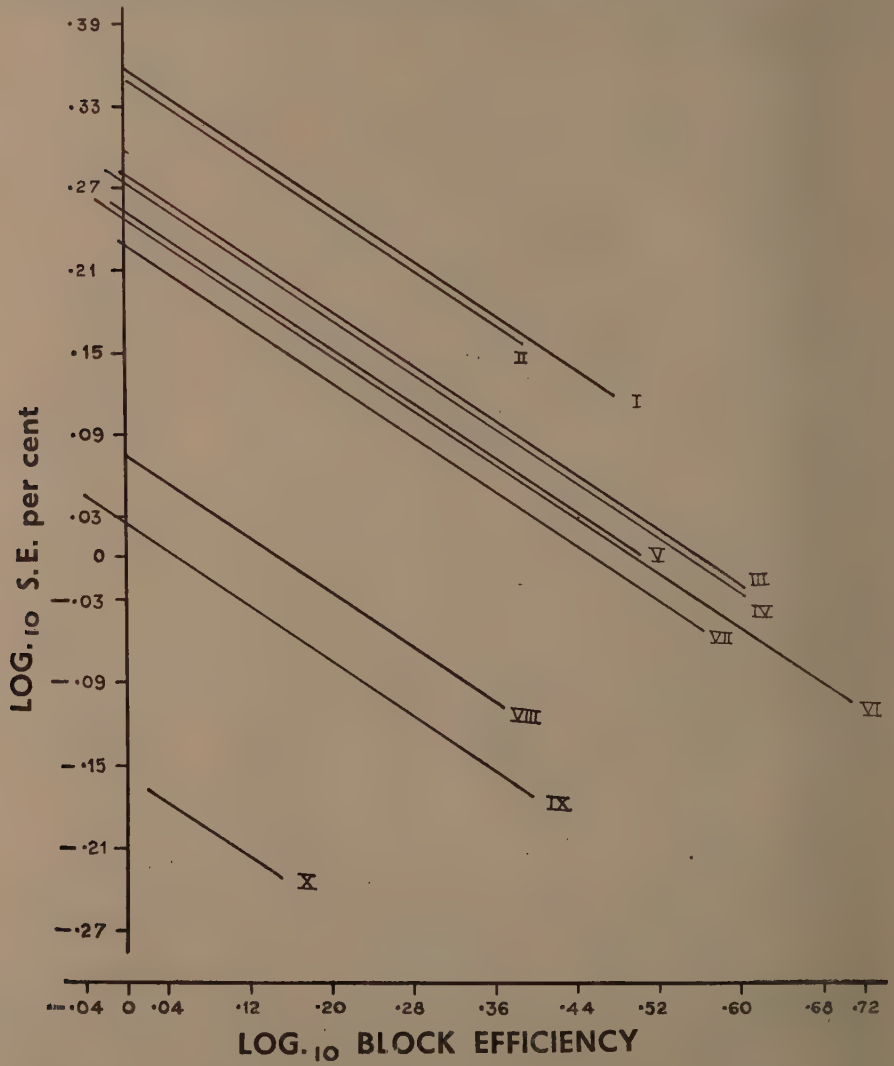

FIG. 2. Relation between logarithms of standard error per cent and of block efficiency

Curve	Plot size (acre)	Plot shape (length : breadth)	Curve	Plot size (acre)	Plot shape (length : breadth
I	1/500	1 : 1	VI	1/100	5 : 1
II	1/500	4 : 1	VII	1/50	2·5 : 1
III	1/200	2·5 : 1	VIII	1/100	20 : 1
IV	1/200	10 : 1	IX	1/50	10 : 1
V	1/100	1·25 : 1	X	1/50	40 : 1

Equations of these straight lines are of the form, $\log y = a - b \log x$, and corresponding equations for the curves drawn in Fig. 1 are,

$$y = 10^a x^{-b},$$

where x and y are block efficiency and standard error per cent respectively. The values of constants a and b can be easily obtained by solving the equations for the straight lines. The value of constant a ranges between 1·3574 and 0·8410, while constant b is approximately 0·5 in all cases. The different lines have thus the same slope or are parallel to one another. This means that for a given change in block efficiency the relative increase or decrease in the magnitude of the standard error is the same for plots of different sizes and shapes.

Constant a represents the logarithm of the standard error per cent when block efficiency is unity, that is when there is no association between neighbouring plots so that the error variance is the same whatever the size and shape of blocks. The ratio of standard errors per cent at this point determines the relative efficiency of blocks necessary to provide the same standard error per cent with plots of different sizes and shapes or the relative magnitude of the standard errors obtained when block efficiency is constant.

In subsequent sections only long and narrow plots of each size will be discussed as being the most efficient.

Number of plots per block

The effect of increasing the size of block on experimental error by including 2, 4, 8, 16, 32 and 64 plots per block may now be considered. Since plots of different sizes and shapes are involved, the relative efficiency of different lay-outs can be conveniently expressed in terms of the number of replications and the amount of land required to give a desired level of accuracy in making experimental comparisons. A standard error of 4 per cent of the mean is adopted as a standard, enabling differences of 11 per cent or more between two varieties or treatments to be relied upon as significant. The results are given in Table III. If a standard error of 2 per cent is aimed at, the number of replications and the amount of land required will be four times that given in Table III.

A similar table for 4-plot and 8-plot blocks was given previously [Hutchinson and Panse, 1935]. The present table shows that for any number of plots per block, plots of a smaller size require more replications but less total area than larger plots to give a 4 per cent standard error. The reason is that the reduction in plot error is not proportional to the increase in plot size. The conclusions to be drawn from this table regarding the choice of plot size are discussed in the paper referred to and will not be repeated here. It is important to notice for our present object that a lay-out containing up to 16 plots per block can give a 4 per cent error with less than ten replications if plots of 1/200 acre size or larger are employed. This number of replications cannot be considered excessive. Even with blocks of 32 plots, plots of 1/100 acre size require only six replications to give a 4 per cent error. Only the smaller plot sizes, 1/500 and 1/200 acre, were available for making combinations containing 64 plots to a block. With these plots at least 20 replications are necessary to attain a 4 per cent error and here the possible advantage of

securing the same standard of accuracy with less replication by resorting to confounding is worth serious consideration. For any number of plots per block, 1/500 acre plots require considerably more replication than other plot sizes and cannot be considered suitable except where the use of such small plots is inevitable.

TABLE III

The number of replications and area of land required to give a standard error of 4 per cent with blocks of different sizes

No. of plots per block	Plot size (acre)	Standard error per cent per plot	No. of replications	Total area (acres)
2	1/500	14·32	13	0·05
	1/200	9·38	5	0·05
	1/100	7·78	4	0·08
	1/50	5·86	2	0·08
4	1/500	14·42	13	0·10
	1/200	9·97	6	0·12
	1/100	8·39	4	0·16
	1/50	6·42	2	0·16
8	1/500	15·61	15	0·24
	1/200	11·41	8	0·32
	1/100	9·50	5	0·40
	1/50	6·77	3	0·48
16	1/500	16·02	16	0·51
	1/200	11·96	9	0·72
	1/100	9·71	6	0·96
	1/50	6·56	3	0·96
32	1/500	18·05	20	1·28
	1/200	14·34	13	2·08
	1/100	9·99	6	1·92
64	1/500	19·28	23	2·94
	1/200	17·75	20	6·40

CONFOUNDING

The gain in efficiency due to confounding is derived from a sub-division of each replicate into smaller blocks. In the analysis of variance, a sum of squares corresponding to differences between sub-blocks is removed from error in addition to the sum of squares due to complete replicates and the error variance is consequently further reduced. The advantage due to confounding will depend upon the magnitude of this reduction in the error variance. Table IV is arranged to compare confounded arrangements with ordinary randomized blocks containing 16, 32 and 64 plots. The gain due to confounding is reflected in the reduced number of replications necessary to give a 4 per cent error. It is more precisely shown by the relative efficiency of the confounded arrangements.

TABLE IV

Relative efficiency due to confounding and number of replications and area of land required to give a standard error of 4 per cent

No. of plots per replicate	Plot size (acre)	Without confounding			Confounded arrangements				Relative efficiency due to confounding
		S. E. per cent per plot	No. of replications	Total area (acres)	No. of plots per sub-block	S. E. per cent per plot	No. of replications	Total area (acres)	
16	1/500	16·02	16	0·51	4	14·42	13	0·42	1·234
	1/200	11·96	9	0·72	4	9·97	6	0·48	1·438
	1/100	9·71	6	0·96	4	8·39	4	0·64	1·339
	1/50	6·56	3	0·96	4	6·42	2	0·64	1·045
32	1/500	18·05	20	1·28	8	15·61	15	0·96	1·336
					4	14·42	13	0·83	1·565
	1/200	14·34	13	2·08	8	11·41	8	1·28	1·579
					4	9·97	6	0·96	2·067
	1/100	9·99	6	1·92	8	9·50	5	1·60	1·105
					4	8·39	4	1·28	1·417
64	1/500	19·28	23	2·94	16	16·02	16	2·05	1·447
					8	15·61	15	1·92	1·524
					4	14·42	13	1·66	1·786
	1/200	17·75	20	6·40	16	11·96	9	2·88	2·200
					8	11·41	8	2·56	2·418
					4	9·97	6	1·92	3·165

The relative efficiency due to confounding is simply the ratio of efficiency of smaller blocks in the confounded arrangement to that of blocks corresponding to a complete replication. Reduction in block size to a quarter or less by confounding has resulted in an appreciable gain in efficiency except only in two cases where it is 10 per cent or less. Plots of 1/50 and 1/100 acre size are involved in these two cases and a reference to Table I will show that with these plot sizes the difference in the efficiency of blocks containing four or more plots and eight or more plots respectively is quite small. Moreover, since with 1/50 acre plots the efficieney of blocks of this size is low, no advantage is to be expected by adopting confounding for this plot size. With 1/100 acre plots the gain in efficiency will be appreciable only when the sub-blocks contain less than eight plots. For the two smaller plot sizes there is a considerable reduction in block efficiency with increasing block size throughout the observed range, and with these plots, confounding has proved more profitable than with larger plots. The gain due to confounding is also naturally greater when there is a larger number of plots in each replicate.

Arrangement of varieties in quasi-factorial or symmetrical incomplete block lay-outs is analogous to confounding, but with one difference. Here we are interested in the comparison of individual varieties and all comparisons are therefore of equal importance. This has the effect of increasing the variance of the comparisons by a factor depending on the number of varieties to be tested and the type of design. This point must be taken into consideration in studying the relative efficiency of varietal trials in incomplete block lay-outs.

For a two-dimensional quasi-factorial arrangement with two equal groups of sets, the mean variance of all comparisons between two varieties is $\frac{2s^2}{r}\frac{p+3}{p+1}$ where s^2 is the error variance, r is the number of replications of each variety and p is the number of varieties in each set [Yates, 1936,1]

This means that the error variance given by this arrangement must be further multiplied by $\frac{p-3}{p-1}$ to compare its efficiency with that of ordinary randomized blocks. The corresponding factor for a three-dimensional design with three equal groups of sets is $\frac{2p^2+5p+11}{2(p^2+p+1)}$. For symmetrical incomplete blocks with p^2 varieties, the factor is $\frac{p+1}{p}$ and a minimum of $p+1$ replications are required [Yates, 1936, 2].

With 16 varieties a two-dimensional quasi-factorial arrangement with four varieties in each block is possible. Sixty-four varieties can be similarly tested in blocks of eight. Sixty-four being a complete cube, a three-dimensional quasi-factorial design is also available. If a symmetrical incomplete block lay-out is used, a minimum of five replications for 16 varieties and nine for 64 varieties will be necessary. The relative efficiency of the two quasi-factorial arrangements was calculated and is given in Table V The number of replications and total area required to give a significant difference of 11 per cent between two varieties, which is equivalent to a 4 per standard error of the mean, are also shown.

TABLE V

Relative efficiency and number of replications required to give a 4 per cent standard error for two and three dimensional quasi-factorial designs

Plot size (acre)	Arrangement	Relative efficiency	No. of replications	Total area (acres)
1/500	4×4 two-dimensional quasi-factorial	0·881	18	0·58
1/200	4×4 two-dimensional quasi-factorial	1·027	9	0·72
1/100	4×4 two-dimensional quasi-factorial	0·956	6	0·96
1/50	4×4 two-dimensional quasi-factorial	0·746	4	1·28
1/500	8×8 two-dimensional quasi-factorial	1·249	19	2·43
1/200	8×8 two-dimensional quasi-factorial	1·982	10	3·20
1/500	4×4×4 three-dimensional quasi-factorial	1·190	20	2·56
1/200	4×4×4 three-dimensional quasi-factorial	2·110	9	2·88

The theoretical loss of efficiency with quasi-factorial arrangements is such that with only 16 varieties the reduced size of block has not been able to overcome the loss for any plot size. The simpler arrangement in ordinary randomized blocks is thus also the more efficient one for testing this number of

varieties. For 64 varieties, on the other hand, the 8×8 arrangement has proved advantageous, particularly with plots of 1/200 acre. The alternative arrangement in $4 \times 4 \times 4$ is not more efficient and cannot be considered suitable for this number of varieties owing to its greater complexity. It may be noted that the number of replications necessary to give a 4 per cent error by adopting a quasi-factorial lay-out when 16 or 64 varieties are under trial, is not less than the minimum required for a symmetrical incomplete block arrangement. The latter besides being slightly more efficient is characterized by simplicity in statistical analysis and equal precision of all comparisons and is therefore to be preferred to quasi-factorial designs in such cases.

Discussion and conclusions

Designs for field experiments have, as their basis, the fact that adjacent areas are more strongly correlated than distant areas. As a consequence of this correlation, plots of a large size and long and narrow shape, small compact blocks and arrangement of plots in one row, particularly with long plots, should be most efficient in reducing experimental error. The results obtained from the analysis of the present uniformity trial are in accordance with this expectation. They are further useful in showing the possible limits up to which advantage of the favourable interaction between the various factors affecting experimental error can be taken in planning experiments under similar conditions.

By applying the method of regression to the analysis of uniformity trial data, Smith [1938] has established an empirical relationship of considerable general interest between plot size and variability. The relationship is of the same form as that shown above between block efficiency and coefficient of variability. In using this relationship to evaluate the optimum size of plots and blocks, shape, however, has been neglected. Smith recognizes the possible influence of plot and block shape on variability ; but his own uniformity trial was too small (the largest plot size was only 1/726 acre) to demonstrate it clearly. It is yet interesting to note that the increased scatter of points with increasing plot size in the diagram in Smith's paper showing the logarithmic relationship between plot size and variability is presumably due to the greater effect of plot shape on variability as plot size increases. An inspection of the table giving variances of plots of different sizes and shapes reveals that for the larger sizes longer plots are generally less variable. Smith's method provides estimates of average relative efficiencies of varying sizes of plots and of blocks ; but in view of the present results these would appear to be of very limited practical value. Changes in the shape of plots and blocks and in the arrangement of plots have been shown to produce considerable variation in the efficiency of any particular size of plot or block, and it does not appear likely that these factors can be subjected to a simple statistical treatment. The somewhat laborious examination of individual combinations of varying sizes and shapes of plots with varying sizes and shapes of blocks remains, therefore, the only means of tackling the problem.

An important point in planning experiments at research stations is the cost of experimental work. This will largely depend on the total area under

experiments. For testing any number of varieties or treatments, with a desired level of accuracy, small plots have been shown to require less total area than large plots, whatever the type of design adopted. For this reason, the smallest size of plot possible in conformity with agricultural requirements would be considered most economical. Against this, however, must be set the increased labour and equipment necessary for handling a large number of replicates. Considering both factors, small plots would seem to be particularly advantageous when a large number of varieties or treatments is under trial. The possibility of increasing experimental accuracy by adopting confounding is another argument in favour of using small plots under these conditions. With plots of a large size, the efficiency of blocks containing even a small number of plots is too low for confounding to be of any advantage. This has been demonstrated for plots of 1/50 acre size.

With the other three plot sizes, the increase in efficiency due to confounding is 20 - 40 per cent when there are 16 plots to a replicate, sub-divided into 4-plot blocks, but with 32 or more plots per replicate and the same size of sub-blocks the increase is 40 per cent or greater. From these results, confounding appears most likely to be profitable when there are 32 or more treatments in an experiment. With only 16 treatments, the relatively smaller gain from confounding would be desirable if differences between treatments are expected to be small, otherwise the simpler lay-out in ordinary randomized blocks would be preferred. In variety trials the gain in efficiency with quasi-factorial arrangements is less than that with an equivalent degree of confounding in agronomic experiments of the same size. In the present analysis the 4×4 quasi-factorial design with only 16 varieties has proved actually less efficient than ordinary randomized blocks. For a higher efficiency with these designs a considerably larger number of varieties should be under trial.

Where quasi-factorial arrangements are appropriate, the symmetrical incomplete block design deserves particular notice. An essential condition for its use is that a certain minimum number of replications is necessary, this number being $p+1$ when p^2 varieties are to be tested. Thus, for 100 varieties, 11 replications will be the minimum. This cannot be considered excessive, since, as we have seen before, nine replications are required for a quasi-factorial lay-out with 64 varieties to give a 4 per cent error of the mean.

Summary

The relation between block size and experimental error is important in planning agricultural trials. A uniformity trial on Malvi cotton was examined to study the question, by combining plots of 1/50, 1/100, 1/200 and 1/500 acre size into blocks of varying sizes.

There is a general decrease of block efficiency with increasing block size. More compact blocks of the same size show a higher efficiency. Blocks of identical size and shape but consisting of long plots also show a somewhat higher efficiency than blocks with short plots of the same size. Arrangement of plots in more than one row decreases block efficiency and the effect is more pronounced with long plots.

A logarithmic relationship is shown to exist between block efficiency and experimental error ; but larger and longer plots give a lower error irrespective

of block efficiency. In determining experimental error plot size and shape are therefore of greater importance than block efficiency.

The number of replications and total area of land required to give a 4 per cent error of the mean were calculated. For the same number of plots per block, smaller plots require more replication but less total area than larger plots.

With all plot sizes except the largest, and with 16 or more plots to a replicate, there is a gain in efficiency by confounding ; but a quasi-factorial arrangement for only 16 varieties was found less efficient than simple randomized blocks.

Factors influencing the choice of design for agronomic and varietal trials of different sizes are discussed.

Acknowledgements

My thanks are due to Mr K. Ramiah for reading through the manuscript and making several useful suggestions.

I acknowledge with thanks the assistance given by Mr V. B. Sahasrabudhe, who is responsible for a large part of the laborious computation.

The investigation was carried out as part of the Cotton Genetics Scheme financed by the Indian Central Cotton Committee.

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APPENDIX

Uniformity trial on Malvi cotton, 1933-34, Institute of Plant Industry, Indore, C. I.

(Yield of seed cotton per plot in gm.)

93	95	111	116	97	82	80	131	80	102	97	95	87	136	118	89	57	106	118	97	72	120	117	117	66	123	116	86	87	79	78	56	1
49	63	50	115	83	78	69	93	85	84	64	69	51	44	115	61	54	54	36	88	62	75	52	91	74	66	76	79	54	54	109	87	2
89	90	95	129	97	133	72	115	97	76	91	106	40	147	119	66	122	63	72	34	47	106	132	97	70	78	56	81	59	81	78	61	3
85	91	115	107	99	77	76	63	70	71	59	78	58	85	61	104	84	104	59	56	70	66	51	83	77	64	87	87	62	80	87	107	4
63	96	68	69	101	120	96	100	70	110	88	75	101	61	106	113	93	62	92	109	84	117	101	75	100	104	129	96	107	116	82	101	5
86	82	118	74	117	28	83	81	109	91	53	108	83	74	129	89	79	89	75	114	106	94	93	71	103	70	63	114	55	92	67	126	6
62	86	119	80	79	92	72	103	89	77	67	88	97	50	101	111	100	96	66	113	98	86	97	69	105	76	87	73	108	104	116	73	7
94	76	119	75	125	96	95	74	90	63	68	66	96	66	85	74	160	148	84	110	110	110	83	154	101	103	89	137	79	111	120	82	8
45	96	115	130	99	77	104	116	64	77	32	88	67	76	72	87	59	123	67	59	113	131	55	101	105	98	98	57	69	78	62	99	9
51	79	111	79	84	84	67	63	66	40	56	80	58	68	52	76	87	72	91	81	153	82	70	133	96	108	68	92	127	91	110	88	10
81	80	72	102	104	81	42	76	85	80	56	74	93	88	60	111	128	124	117	118	77	84	104	105	119	101	104	129	53	62	99	75	11
74	51	66	86	71	73	98	87	61	111	81	61	73	61	63	85	115	80	82	76	86	97	65	75	68	98	67	69	75	89	72	88	12
85	89	112	88	105	76	76	78	72	92	59	81	90	52	82	68	88	94	68	106	87	106	80	88	71	85	82	108	104	98	95	90	13
84	64	101	90	69	101	87	70	63	75	63	88	74	81	68	87	82	80	64	105	67	70	58	79	88	58	73	53	85	64	96	86	14
85	55	88	87	73	119	60	76	60	73	77	44	83	82	74	52	97	65	59	75	110	79	101	74	66	84	87	100	67	83	61	70	15
82	83	102	80	88	57	81	55	75	69	70	84	59	73	77	81	98	74	95	110	119	93	95	81	102	82	74	82	80	75	85	99	16
46	54	83	60	57	75	73	50	66	31	56	66	43	70	59	35	90	62	89	96	87	72	80	88	67	67	107	69	79	48	89	59	17
71	68	39	80	73	86	64	89	101	88	63	83	74	78	74	50	126	98	104	109	45	106	107	100	137	110	102	79	134	45	60	78	18
58	52	85	99	72	96	67	86	37	71	62	48	63	64	106	90	91	86	83	104	55	70	73	67	67	50	91	88	89	70	56	66	19
65	45	77	78	93	60	94	82	72	81	84	64	75	97	114	109	111	74	107	86	53	52	90	53	72	70	43	53	106	49	67	62	20
57	52	60	71	55	89	71	53	64	74	95	59	68	94	85	90	98	94	84	62	60	54	50	35	64	70	54	62	85	77	78	76	21
60	90	65	66	59	55	51	79	80	62	95	94	95	96	114	105	113	49	123	104	60	71	83	81	79	50	69	76	111	79	108	119	22
59	69	39	79	74	68	54	72	69	67	81	72	56	68	92	62	103	89	98	64	115	41	73	99	78	72	104	56	121	80	128	157	23
63	78	60	83	51	60	60	72	75	45	75	69	93	89	75	87	114	147	138	118	106	106	86	85	95	154	78	98	133	125	133	101	24
23	67	47	83	74	58	95	108	81	86	88	99	82	90	133	97	85	113	137	82	130	82	106	96	53	102	103	82	124	108	116	108	25
39	57	83	70	77	89	79	113	77	102	101	74	79	97	100	41	77	98	71	98	102	75	77	83	112	106	95	106	93	68	94	103	26

79	29	57	77	75	82	87	77	61	85	108	65	64	95	56	81	153	116	45	132	103	88	94	123	76	145	103	107	122	94	108	121	27
81	54	74	87	71	74	62	62	90	80	83	60	68	53	82	107	152	40	80	124	130	105	103	69	75	115	138	129	165	135	143	138	28
56	47	86	69	81	60	73	96	73	59	83	83	89	130	74	76	133	30	43	81	96	85	86	140	103	92	88	93	97	38	82	103	29
52	78	62	55	60	58	111	54	87	76	82	103	114	92	70	39	87	35	46	39	52	157	80	63	59	69	63	72	64	68	102	62	30
57	70	68	87	79	89	87	91	85	74	67	98	89	69	50	57	56	54	71	70	69	92	47	41	29	52	46	79	87	74	73	65	31
44	41	81	80	90	78	61	76	78	87	106	41	49	61	70	78	145	40	79	103	71	29	51	28	30	37	37	53	49	81	28	36	32
59	68	44	71	75	79	64	68	62	118	60	78	63	53	67	51	84	40	64	46	34	15	33	56	32	35	42	37	35	97	56	66	33
48	33	65	85	64	74	96	74	95	101	56	59	61	77	57	44	88	91	29	50	40	32	61	47	8	27	54	72	76	67	89	99	34
69	63	68	71	54	59	84	71	84	87	82	73	73	73	48	42	28	36	31	13	17	35	18	24	14	22	33	42	52	82	60	72	35
59	66	95	74	81	77	94	106	132	80	142	30	83	46	56	29	82	25	41	30	23	46	47	35	40	58	63	61	63	92	57	78	36
72	51	66	48	65	82	119	101	90	53	69	66	45	54	61	68	47	44	32	32	50	37	32	53	17	30	43	5	42	36	62	61	37
81	69	58	66	45	77	94	84	98	44	57	58	17	68	45	39	82	61	33	72	65	86	37	57	53	44	47	56	76	56	75	87	38
61	106	43	84	46	74	56	109	115	61	60	39	66	61	57	37	109	71	81	52	79	92	45	62	49	87	111	73	93	58	56	51	39
83	87	69	64	76	82	61	64	87	59	99	31	81	45	50	31	52	40	50	53	57	97	52	81	82	76	54	55	91	12	50	80	40
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	

VARIATION IN THE MEASURABLE CHARACTERS OF COTTON FIBRES

III. VARIATION OF MATURITY AMONG THE DIFFERENT REGIONS OF THE SEED SURFACE

BY

R. L. N. IYENGAR, M.Sc.

Agricultural Research Institute, Coimbatore

(Received for publication on 12 November 1940)

Introduction

I

THE halo obtained by combing the fibres on a seed cotton, when observed closely, reveals that the fibres are not uniformly distributed over the entire seed surface. Near the funicle the fibres are few while at the chalazal end they are very densely populated. It has been shown by many workers that the fibres at the latter region are the first to form. These differences in the time of formation and the density of fibre population—leaving alone the changes in the position of vascular strands—may be expected to influence the physical characters of the fibres produced at the different regions of the seed surface. Koshal and Ahmad [1932] have made a detailed study on this point in regard to the properties—length, weight and strength. They find considerable variations in different regions, more especially at the chalazal end. A small study by the writer [Iyengar, 1932] on one cotton indicated somewhat similar differences in length, weight and maturity. The present work is an extension of it, particular attention being paid to the variation of maturity in the different regions. The study has indicated that the immaturity is considerable at the chalazal end. In order to see whether any manurial treatment would be able to mitigate this low maturity, the work was extended to samples obtained from different manurial treatments. The conclusions drawn in this case, however, should be considered tentative as they are derived from a single cotton.

Material and method

Fourteen pure strains of cotton formed the material for this enquiry They were all grown in field No. C 1 of the Cotton Breeding Station, Coimbatore, during the season 1932-33. Turner [1929] has dealt with the various factors that induce variability in cotton. In the present enquiry the effects of some of these factors, like the date of picking, the composition of the boll and the lock and the position of the seed in the lock, were minimized by sampling in the manner described below. Single day's picking was taken, only three-locked bolls being picked in all cases except for Verum 262, Gadag 1 and Co 2 where four-locked bolls were picked. From them the middle seed of 7-seeded locks (9-seeded for Co2, Verum 262 and Mollisoni ; 8-seeded for Roseum and

6-seeded for 171 and Chandajari) was taken to make up the sample. The number of seeds in a sample ranged from 30 to 60 in the different varieties. The seeds were carefully combed to form a full halo, care being taken to pull out as few hairs as possible. The ' combed waste ' was also preserved and examined separately.

The surface of the seed was divided into six regions : (1) micropylar end, (2) portion adjacent to the raphe, (3) right side*, (4) left side, (5) back of the seed (that is, antipodes to the raphe) and (6) chalazal end. In order to avoid overlapping, fibres in the borderland were discarded ; from the combed halo, a tuft of fibres sprouting from the region required was separated and pulled out by means of a dissecting needle. Tufts from the same region of other seeds were put together and made into a sliver for the study of the maturity. Clegg's [1932] method was employed and ten tufts of about 100 fibres each were tested for each region.

For the subsidiary study on the effect of manurial treatment, only two regions—the micropylar and chalazal ends of the seed—were considered. The material was of Co 2 obtained from plots laid out for the study of the effects of different mineral manures. Five treatments, namely (1) no manure, (2) N (nitrogenous manure), (3) N and K (nitrogen and potash), (4) N and P (nitrogen and phosphoric acid) and (5) N, K and P (nitrogen, potash and phosphoric acid) were given in each of two fields, 5-b with rich soil, and 3-b with poor soil. Two sets of samples were taken from the two fields by picking on two different dates, *viz.* 28 February 1931 and 7 March 1931. The fifth seed of 9-seeded locks from 4-locked bolls made up the samples. The fibres from the micropylar and chalazal ends only were pulled out as described above and tested for maturity. Incidentally the mean length and the weight per cm. for these samples were determined in order to have an idea of the effect of the manurial treatments on these characters also.

The statistical significance of the differences has been found as follows. In the 14 strains, for the mature and immature fibre percentages, the standard error for each region was calculated by the formula $\sqrt{\frac{pq}{n}}$, where p and q represent the percentages of fibres falling within and outside the particular class under study and n the total number of fibres examined. The standard error of the difference between two regions is computed as $\sqrt{s_1^2+s_2^2}$, s_1 and s_2 being the standard errors for the two respective regions. In the study of the effect of manures analysis of variance was employed.

Results

Fourteen samples

The results for the variation of the mature and immature fibres in the different regions are given in Table I. The percentages of mature fibres may first be considered. It will be seen that at the micropylar end the fibres are very mature, 99 per cent of them being mature in some cottons, the lowest being 90 per cent in the case of Jayawant. The chalazal end exhibits considerable variations in the different strains. Though strains like Roseum,

* The halo is placed so that the raphe faces the observer and the micropylar end points upwards.

TABLE

Maturity in the different

No.	Species	Strain	Mature fibres percentage											
			Micropylar		Chalazal		Right		Left		Raphe		Back	
			Value	S. E.	Value	S. E.	Value	S. E.	Value	S. E.	Value	S. E.	Value	S. E.
1	*G. arboreum.*	Roseum	99·1	0·27	86·2	1·03	98·0	0·42	96·4	0·57	96·8	0·50	96·4	0·57
2	,,	Mollisoni	95·8	0·57	88·5	0·92	96·0	0·56	95·5	0·61	95·0	0·62	91·7	0·82
3	,,	Cocanadas 171	97·7	0·44	76·7	1·28	97·1	0·51	96·9	0·53	95·6	0·61	94·6	0·67
4	,,	Chandajari	98·9	0·30	69·2	1·35	96·3	0·57	90·5	0·51	97·5	0·45	96·6	0·51
5	,,	*Sanguineum*	97·9	0·42	61·5	1·37	96·4	0·54	93·5	0·72	93·0	0·74	90·0	0·86
6	,,	N 14	95·5	0·59	63·3	1·34	95·5	0·58	89·0	0·89	92·0	0·74	95·0	0·57
7	,,	Bani 306	97·9	0·43	47·2	1·37	90·6	0·84	91·3	0·71	81·2	1·09	89·4	0·84
8	,,	Karunganni C 7	98·8	0·32	34·1	1·40	88·7	0·91	89·0	0·94	94·4	0·67	83·9	1·06
9	,,	Verum 262	95·6	0·56	28·2	1·18	88·4	0·89	87·1	0·89	90·8	0·81	87·3	0·97
10	*G. herbaceum.*	H 1	95·9	0·59	91·7	0·77	96·1	0·57	96·5	0·55	97·1	0·49	91·8	0·79
11	,,	2405	97·4	0·46	83·0	1·05	92·7	0·77	95·3	0·64	95·3	0·57	93·8	0·72
12	,,	Jayawant	90·1	0·84	69·8	1·36	85·7	1·06	88·6	0·93	91·6	0·78	82·5	1·05
13	*G. hirsutum*	Gadag 1	97·6	0·42	85·5	1·00	96·3	0·53	96·8	0·48	97·6	0·42	94·0	0·48
14	,,	Co 2	94·1	0·76	36·6	1·48	77·9	1·21	67·6	1·48	79·1	1·22	77·4	1·34

I

regions of the seed surface

Immature fibres percentage

Waste		Micropylar		Chalazal		Right		Left		Raphe		Back		Waste	
Value	S. E.	Value	S. E.	Value	S. E.	Value	S. E.	Value	S. E.	Value	S. E.	Value	S. E.	Value	S. E.
85·0	1·09	0·2	0·13	2·6	0·47	0·4	0·19	0·5	0·21	0·3	0·16	0·5	0·21	5·9	0·72
74·0	1·30	0·9	0·27	3·5	0·53	0·5	0·20	0·7	0·24	0·9	0·27	1·9	0·41	10·5	0·91
72·0	1·33	0·5	0·21	11·8	0·97	1·0	0·31	1·1	0·32	1·8	0·39	1·7	0·38	18·0	1·13
51·5	1·46	0·2	0·13	10·7	0·90	1·2	0·33	1·6	0·35	0·6	0·22	1·2	0·30	30·4	1·35
73·3	1·30	0·4	0·18	5·2	0·64	0·5	0·21	1·4	0·34	1·2	0·32	2·6	0·46	10·0	0·78
58·5	1·32	0·8	0·25	14·8	0·99	0·8	0·25	5·2	0·63	3·0	0·46	1·3	0·30	30·1	1·23
47·3	1·39	0·9	0·28	31·8	1·12	5·2	0·64	4·5	0·62	11·4	0·88	4·5	0·57	33·8	1·33
41·5	1·47	0·3	0·16	36·4	1·42	4·2	0·58	3·8	0·57	1·7	0·37	6·1	0·69	39·1	1·45
51·4	1·35	0·7	0·23	33·2	1·24	2·7	0·45	3·8	0·51	2·4	0·43	4·3	0·59	27·6	1·21
85·9	1·02	0·9	0·28	22·4	0·43	0·5	0·21	0·7	0·25	0·9	0·27	2·9	0·47	4·4	0·60
83·0	1·12	0·3	0·18	5·1	0·61	1·2	0·32	0·6	0·23	0·8	0·24	1·1	0·31	3·4	0·54
53·0	1·43	1·8	0·38	9·8	0·88	4·5	0·61	2·9	0·49	2·6	0·45	6·2	0·67	18·2	1·11
74·2	1·26	0·1	0·09	1·0	0·27	0·3	0·15	0·4	0·17	0·2	0·12	0·4	0·17	4·2	0·58
45·8	1·54	2·1	0·46	39·8	1·50	10·4	0·89	15·9	1·16	12·7	1·00	9·7	0·92	42·7	1·53

Mollisoni, H 1, 2405 and Gadag 1 have fairly high percentages, there are others which have very small percentages. For example Verum 262 records the lowest figure of 28 per cent, Karunganni C 7 has 34 per cent, while Co 2 has 37 per cent. In all the strains this region has a significantly lower figure than the micropylar end.

The other four regions may be considered together, for though some of the differences are statistically significant, generally speaking the variation among these four regions, within a strain, is not large. The raphe region of Bani 306 and the back region of Karunganni C 7 and Jayawant exhibit significantly lower maturity than the others of the four regions of the respective strains. For N 14 the left side indicates a lower maturity than the right and back regions. In Co 2 the left side is significantly less mature than the raphe and back regions. In view of the negligibility of the differences between the right and left sides in all the others, except N 14, this huge difference observed is rather surprising.

When the maturity of these regions is compared with that for the chalazal end all differences, except one (H 1 back region), are statistically significant. When compared with that for the micropylar end, Bani 306, Karunganni C 7, Verum 262 and Co 2 exhibit significantly lower percentages. In other cases the differences are not significant.

When the maturity figures of the combed waste are considered, it will be seen that generally they are nearly the same as for the chalazal end, though in some cases it is more and in some others less.

The values for the immature fibres may now be considered. It will be seen that the variations of this figure corroborate the statements made above according to the mature fibres, and therefore need not be repeated. In this case also the left side of Co 2 indicates lesser maturity than the right side and back, though not as low a maturity as was exhibited by the mature fibres.

Summing up the above, it may be stated that: (1) the fibres at the micropylar end are very mature; (2) at the chalazal region the maturity varies considerably in the different strains; the maturity for this region is, however, significantly less than that for the other regions in all cases except one; (3) among the other four regions the differences are generally not considerable; in only four strains is the maturity of these regions significantly less than that for the micropylar end.

Effect of manurial treatment

It has been found above that generally it is the chalazal end that contains high percentage of immaturity. It was thought valuable to study if any manurial treatment would enable this region to improve its maturity. Samples from plots obtaining different mineral manurial treatments which were available were utilized for this enquiry. It should be mentioned at the outset that the conclusions drawn in the following should be considered tentative only on account of (1) the results being obtained for only one cotton and (2) the number of replications and consequently the number of degrees of freedom for the error variance being not large. The results are recorded in Table II. It will be seen that the micropylar end records high percentages with very small variations. At the chalazal end, however, the differences are fairly large in some cases. In the rich field (5-b) the differences between the treatments are very small for the picking of 28 February excepting for the high value 34 per

cent for treatment N. For the other picking of 7 March apparently a small improvement is indicated with the supply of better nutriment, but the differences are small and not significant. In the poor field (3-b), on the other hand, there is a significant improvement of maturity with improved plant food in both the pickings. In view of this trend it is rather surprising why the better nutrition available in the rich field has produced lower maturity (28·6), especially in the picking of 28 February. The cause remains unknown.

To assess the statistical significance of the maturity, in this case, the maturity ratio of Peirce and Lord [1939] would be the appropriate measure. It takes into account the percentages of the three maturity classes and expresses the maturity as a single factor. These values also are given in Table II and the statistical analysis of them is found in Table III.

TABLE II

Variation of maturity with manurial treatment

Property	Treatment	Micropylar end					Chalazal end				
		5-b		3-b		Mean	5-b		3-b		Mean
		28-2	7-3	28-2	7-3		28-2	7-3	28-2	7-3	
Mature fibres (per cent)	No manure	92	96	97	97	96·0	28	33	24	31	29·0
	N	98	94	96	96	95·5	34	34	32	35	33·8
	N + K	96	96	97	96	96·2	26	35	30	37	32·0
	N + P	96	97	96	98	96·8	28	36	39	41	36·0
	N + K + P	96	95	98	98	96·8	27	37	43	46	38·0
	Mean	95·6	95·6	96·8	97·0	96·3	28·6	35·0	33·6	38·0	33·8
	Fields	95·6		96·9			31·8		35·8		
	Pickings		96·2	96·3				31·1	36·5		
Immature fibres (per cent)	No manure	0·9	0·7	0·7	0·5	0·7	24	24	26	25	24·8
	N	0·4	0·6	0·6	0·6	0·6	19	21	21	23	21·0
	N + K	0·5	0·8	0·8	0·4	0·6	25	20	21	21	21·8
	N + P	0·6	0·7	0·3	0·4	0·5	24	24	22	18	19·5
	N + K + P	1·0	0·4	0·2	0·2	0·4	20	19	22	17	19·5
	Mean	0·7	0·6	0·5	0·4	0·6	22·4	21·6	22·4	20·8	21·8
	Fields	0·6		0·4			22·0		21·6		
	Pickings		0·6	0·5				22·4	21·2		
Maturity ratio	No manure	1·158	1·174	1·184	1·180	1·174	0·719	0·744	0·692	0·726	0·720
	N	1·186	1·166	1·176	1·178	1·176	0·776	0·769	0·754	0·758	0·764
	N + K	1·178	1·174	1·180	1·178	1·178	0·705	0·774	0·744	0·780	0·751
	N + P	1·180	1·182	1·178	1·188	1·182	0·720	0·760	0·786	0·815	0·770
	N + K + P	1·178	1·173	1·188	1·190	1·182	0·736	0·790	0·806	0·844	0·794
	Mean	1·176	1·174	1·181	1·183	1·178	0·731	0·767	0·756	0·785	0·760
	Fields	1·175		1·182			0·749		0·770		
	Pickings		1·179	1·178				0·744	0·776		

For the micropylar end*, it will be seen, none of the variances are significant. For the chalazal end, however, all the main effects are highly significant besides the first order interaction between field and treatment, indicating that the fields respond differently to the manurial treatments. In the rich field the influence of nutrition is not significant but in the poorer field maturity responds significantly to it, better nutrition causing increased maturity. The difference between the two fields is also significant, the poorer field recording greater maturity, which is rather irreconcilable with the above findings. The picking of 7 March is significantly more mature than that of 28 February.

It will be seen that for the chalazal end the second order interaction is small and according to that all the main effects and the first order interaction between M and F are highly significant. For the study of the main effects the non-significant interactions, M × P and F × P, may be combined with the second order interaction. Even then all the three main effects are significant. If, however, the main effects M and F are compared with their interaction M × F, both of them are non-significant.

II

The results obtained from the incidental study of the length and fibre weight may now be considered.

Only the mean values of the length, weight per cm. and standard fibre weight are given in Table IV for the sake of brevity and the analysis of variance is found in Table III. The figures for the chalazal end alone have been analysed, as greater variability was exhibited by that region, as was found in the case of the maturity.

Before taking up the differences caused by the treatments, the differences between the two regions may be considered. It will be seen that the length at the chalazal end is always greater than at the micropylar end, the difference being about 10·1—13·7 per cent of the former, similar to that got by Koshal and Ahmad [1932].

The fibre weight per cm. is much less at the chalazal end than at the micropylar end as was found by Koshal and Ahmad [1932]. The increase found in the present cotton, Co 2, is, however, higher than what was obtained by them, being as high as about 120 per cent. The low weight at the chalazal end is seen to be accompanied by greater immaturity, whose influence may be eliminated by calculating the standard fibre weight [Peirce and Lord, 1939]. The results for this character, given in Table IV, show that this also is less at the chalazal end. The increase at the micropylar end over that at the chalazal end is about 40 per cent. What was 120 per cent in the case of the fibre weight per cm. has been reduced to 40 per cent, thus indicating that the low fibre weight was partly due to the immaturity and partly to intrinsic fineness of the fibres at the chalazal end. This supports the findings of the writer made in the study of the cell diameter of the uncollapsed fibre, where the chalazal region recorded a significantly lesser diameter than the micropylar

* The analysis of variance has been carried out separately for the two regions, as it would be incorrect to combine them because the variances for the two regions are not nearly equal or of the same order.

region. The average for 17 *hirsutum* cottons was $24\cdot7\pm0\cdot32\mu$ for the micropylar region and $21\cdot0\pm0\cdot26\mu$ for the chalazal region. For the cotton Co 2 it was found to be $26\cdot4\pm0\cdot52\mu$ and $21\cdot8\pm0\cdot34\mu$ respectively for the two regions.

Table III

Analysis of variance ; mean square and significance

Variance due to	Degrees of freedom	Maturity ratio: Micropylar end		Maturity ratio: Chalazal end		Mean fibre length in inch		Fibre wt. per cm. in. 10^{-6} gm.		Standard fibre wt. in 10^{-6} gm.	
		10^{-3}		10^{-3}		10^{-2}		10^{-2}		10^{-2}	
Manures (*M*)	4	0·0573	N	2·954	HS	0·052	N	0·066	N	1·459	N
Fields (*F*)	1	0·2651	N	2·282	HS	0·013	N	1·305	N	0·336	N
Pickings (*P*)	1	0·0135	N	5·219	HS	0·841	S	1·109	N	0·030	N
Interaction											
$M \times F$	4	0·0443	N	1·627	HS	0·046	N	0·352	N	0·875	N
$M \times P$	4	0·0352	N	0·429	N	0·044	N	0·324	N	0·506	N
$F \times P$	1	0·0090	N	0·045	N	0·004	N	0·080	N	0·014	N
$M \times F \times P$	4	0·0628		0·093		0·046		0·433		0·562	

N = Not significant.
S = Significant for $P = 0\cdot05$
HS = Significant for $P = 0\cdot01$

Table IV

Difference between the micropylar and chalazal ends

Treatment	Mean fibre length in inch			Fibre weight per cm. in 10^{-6} gm.			Standard fibre weight per cm. in 10^{-6} gm.		
	M	C	$\frac{C\sim M}{C}\times100$	M	C	$\frac{C\sim M}{C}\times100$	M	C	$\frac{C\sim M}{C}\times100$
Manures									
No manure	0·85	0·98	13·3	2·92	1·28	128	2·48	1·77	40·1
N	0·89	1·00	11·0	2·82	1·27	122	2·40	1·66	44·6
N + K	0·90	1·02	11·7	2·82	1·26	124	2·39	1·68	42·8
N + P	0·87	1·00	13·0	2·88	1·26	129	2·44	1·63	49·7
N + K + P	0·89	0·99	10·1	2·84	1·29	119	2·40	1·62	48·2
Fields									
5-b	0·87	1·00	13·0	2·86	1·24	130	2·44	1·66	47·0
3-b	0·88	1·00	12·0	2·86	1·30	120	2·41	1·68	43·5
Pickings									
28 February 1931	0·88	1·02	13·7	2·86	1·24	131	2·43	1·68	44·6
7 March 1931	0·88	0·98	10·2	2·86	1·29	122	2·42	1·67	44·8
Grand mean	0·88	1·00	12·0	2·86	1·26	127	2·42	1·68	44·0

M = Micropylar end ; C = Chalazal end

The variances may now be considered. These are found in Table III It will be seen that all the variances are insignificant, except that for length between pickings. The picking of 28 February indicates a significantly longer length than that of 7 March. All the other differences are insignificant. Neither the manures, nor the field of growth has any marked effect on the length, weight per cm. or the intrinsic fineness.

It is beyond the scope of the present work to consider in detail the effect of manurial treatments. Some findings of the workers on the subject may, however, be given. Nelson and Ware [1932] who made an extensive study of the effect of nitrogen, potash and phosphoric acid found no effect of any of these manures on the staple length. Armstrong and Bennett [1933] found that small plants grown on plots of low fertility and clearly suffering from malnutrition produced lint of practically the same length as that produced by vigorous plants growing in plots of high fertility, though the uniformity of distribution of different lengths was less in the poorly nourished plots. Reynolds and Killough [1933] found no effect of nitrogen and potash but an increase in length ' which approached significance ' by phosphoric acid. They concluded that ' while some fertilizers appeared to produce significant increases or decreases in the length of lint, it is probable that these differences, though statistically significant in some cases, are not large enough to be detected in the commercial classing of cotton '. Reynolds with Stansel [1935] failed to establish any definite relation between lint length and manure. Wood [1934] found that in the potash starved soils the lint was shorter, more irregular and contained a larger proportion of poorly thicked fibres. Crowther [1938] ' knew of no case where applying nitrogenous fertilizers had affected the quality of cotton grown under rainfall ' in India. In the Sudan, however, under irrigation nitrogenous fertilizers delayed the maturity of the crop and consequent on the lateness the manured crop ' tended to be inferior in quality to the unmanured crop '.

It can be gathered from the above that though a few workers have found some small influence of some manures, generally speaking, the effect is not large. The non-significant effect observed in the present case falls in a line with these findings.

Conclusions

The following deductions may be drawn from the present findings.

1. The micropylar end contains a very high percentage of mature fibres.

2. The maturity at the chalazal end varies considerably in the different strains. In some strains it is as high as about 90 per cent while in some as low as 30 per cent. In all cases, except one, the maturity for this region is statistically significantly less than that for any of the other regions.

3. In the other four regions studied, namely, right side, left side, region near the raphe and the back side, the differences among one another are generally not significant and the maturity is fairly high in all strains. In only four cottons, it is significantly less than that for the micropylar end.

4. Supply of better nutrition to the cotton plant does not appear to have produced any effect on the maturity in the field in which the soil is rich, while in the field with poor soil, supply of better nutriment is accompanied by improved maturity.

5. Differences in the nutrition supplied appear to have negligible influence on the fibre length, fibre weight per cm. and intrinsic fineness.

ACKNOWLEDGEMENTS

The writer's thanks are due to Rao Bahadur V. Ramanatha Ayyar for suggesting the problem and his interest in this work and to Dr Nazir Ahmad for his valuable suggestions. He is indebted to the fieldmen of the Cotton specialist's section for combing the large number of seeds. The work was carried out under the financial assistance of the Indian Central Cotton Committee.

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A NOTE ON THE VARIATION IN THE STANDARD FIBRE WEIGHT OF THE COTTON FIBRE IN RELATION TO ITS LENGTH

BY

R. L. N. IYENGAR, M.Sc.

Cotton breeding Station, Coimbatore

(Received for publication on 12 May 1941)

IN a previous study by the writer and Turner [1930], it was found that the fibre weight per centimeter varied considerably in the different length grades of the fibres in strains of *G. hirsutum* but in those of *G. arboreum* the variation was not considerable. Similar non-variability was found for Sakel (*G. barbadense*), by Morton [1928] and by Balls [1928]. In another investigation, the writer [Iyengar, 1939] found that the mean ribbon-width and the swollen diameter of the fibre decreased with the increase in length in almost all cases. In the same work the maturity was found to vary in the different lengths, the trend of variation, however, being different in the different strains. As the fibre weight would be influenced by the maturity of the fibre, it was thought advisable to eliminate the influence of the maturity. This was done in the present study by calculating the standard fibre weight data. The results for the fibre weight per centimeter and the maturity percentage from which the standard fibre weight was calculated were taken from another paper [Iyengar, 1941]. For the sake of easy reference they are given in Table II. The formula* of Peirce and Lord [1934] was employed to calculate the standard fibre weight. As some differences were observed between the values for the thin-walled and 'dead' fibres as obtained by the method described by Peirce and Lord [1934] and that given by the writer [Iyengar, 1939], the standard fibre weight was calculated according to both methods. The differences between them are, however, seen to be not considerable.

The results are given in Table I.

It will be seen that in all the three cottons the standard fibre weight systematically increases with decrease in length. In Co 1 and Co 2 the differences between the extreme values of the observed fibre weight per centimeter were 17·2 per cent and 20·2 per cent respectively. These were reduced to 14·0 per cent and 12·2 per cent respectively in the standard fibre weight. In the case of K 546, however, it increased from 9·4 per cent to 22·6 per cent. In other words, it means that in the first two cottons the variations in the maturity showed exaggerated variations in the fineness among the different length grades, while in the third they had a masking effect.

* From a discussion at Technological Assistants' Conference (1940) at the Matunga Laboratory where work on this subject is in progress, it was learnt that the old formula was more suited to Indian cottons than the new one [Peirce and Lord, 1939].

TABLE I

Observed and standard fibre weights

Group length in 1/8 in.	Co 1*			Co 2*			K 546*		
	Observed	Standard		Observed	Standard		Observed	Standard	
		Peirce & Lord	Iyengar		Peirce & Lord	Iyengar		Peirce & Lord	Iyengar
10	1·85	1·90	1·85	1·80	2·08	2·02	2·19	2·01	2·00
9	1·93	1·98	1·94	1·93	2·12	2·05	2·35	2·18	2·17
8	2·02	2·06	2·00	2·05	2·19	2·13	2·40	2·28	2·26
7	2·12	2·11	2·07	2·15	2·27	2·21	2·41	2·40	2·36
6	2·20	2·16	2·13	2·21	2·34	2·28	2·39	2·56	2·51
Difference between extremes as per cent of mean	17·2	12·6	14·0	20·2	11·7	12·2	9·4	24·1	22·6

* Co 1 and Co 2 belong to *G. hirsutum* Linn., K 546 belongs to *G. arboreum* var *neglectum* forma *indica* H. & G.

But when they were expressed in terms of standard fibre weight, where the differences in the maturity were eliminated, all the three strains showed increases with the decrease in length of the fibre. Such a finding is in conformity with the conclusions arrived at from the data of width measurements [Iyengar, 1939].

The above studies demonstrate clearly that the presence of immature fibres has been the cause of the differential behaviour observed in different cottons, in the relationship between the fibre weight and lint length grades.

The foregoing findings lead one to suspect whether the maturity factor may not be the cause for non-variability observed in *G. arboreum* by Iyenger and Turner [1930] and in Sakel both by Morton [1928] and by Balls [1928]. Unfortunately the maturity figures for the different length-grades are not available for these cottons.

TABLE II

Property	Name of strain	No. of determinations	Group length classes in inches					Critical difference ($P = 0·05$)
			10/8	9/8	8/8	7/8	6/8	
Fibre weight per cm (in 10^{-6} gm.)	Co 1	72	1·85	1·93	2·02	2·12	2·20	0·0252
	Co 2	72	1·80	1·93	2·05	2·15	2·21	0·0286
	K 546	56	2·19	2·35	2·40	2·41	2·39	0·0350
Mature fibres (per cent)	Co 1	90	64·7	65·0	66·2	71·8	74·9	3·6
	Co 2	24	47·8	54·1	59·1	62·0	62·5	10·3
	K 546	28	91·9	88·1	83·5	74·2	63·3	5·2
Immature fibres (per cent)	Co 1	90	15·1	15·9	14·8	12·0	11·0	2·5
	Co 2	24	33·4	25·0	21·2	20·2	21·6	8·7
	K 546	28	3·8	4·6	8·0	14·6	25·2	5·9
Half mature fibres (per cent)	Co 1	90	20·2	19·1	19·1	16·2	14·1	2·9
	Co 2	24	18·8	21·0	19·7	17·8	15·8	3·5
	K 546	28	4·4	7·3	8·5	11·2	11·4	3·8

For the analysis of variance of the results reference may be made to the work of Iyengar [1941].

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STUDIES ON THE ROOT-ROT DISEASE OF COTTON IN THE PUNJAB

XI. EFFECT OF MIXED CROPPING ON THE INCIDENCE OF THE DISEASE

BY

R. SAHAI VASUDEVA, B.Sc., PH.D. (LOND.), D.I.C. (LOND.)

*Assistant Plant Pathologist, Section of Mycology and Plant Pathology, Imperial Agricultural Research Institute, New Delhi**

(Received for publication on 21 April 1941)

(With Plates LIX and LX and two text-figures)

THE growing of cotton mixed with another crop has been practised for long in India and other tropical countries. In the Punjab a fodder or a pulse crop is usually sown with cotton. In the Tanganyika territory this has been a native method of introducing simultaneous rotation [Robertson, 1938].

Some preliminary observations on the effect of mixed cropping on the incidence of cotton root-rot disease in the Punjab have already been recorded [Vasudeva and Ashraf, 1939]. It has been shown that the incidence of the disease is significantly reduced in plots in which cotton is sown in mixture with sorghum. Further experiments have now been carried out on similar lines, and the results are presented in this paper.

EXPERIMENTAL

The experiments reported here were conducted at Lyallpur on land which was heavily and uniformly infected with the disease and whose previous history was known from observations made for a number of years. Some experiments were also carried out at the British Cotton Growing Association Farm, Khanewal, in order to confirm the results obtained at Lyallpur.

Cotton was sown in May, which is the optimum time for the occurrence of the disease so that all the necessary conditions were provided for a vigorous attack of the disease in order to obtain reliable data.

A. *Effect of inter-cropping cotton with sorghum*

A plot of land heavily and uniformly infected with the disease was divided into 20 sub-plots 45ft. × 18ft. and sown with *G. indicum* variety Mollisoni 39, an indigenous type, on 16 May 1939. Six rows of cotton were sown in each plot at a distance of $2\frac{1}{2}$ feet apart. Sorghum variety J 20 was sown by broadcasting the same day in between the cotton lines in 16 plots. A border of 2 ft. was also sown with sorghum around the cotton crop so that the plot was surrounded by it on all the four sides. The remaining four plots were sown with cotton only to serve as controls.

*Lately Assistant Cotton Mycologist, Punjab Agricultural College and Research Institute, Lyallpur.

TABLE

Root-rot mortality in mixed

Week ending	Un-cropped area						Cotton				
	8 A.M.			5 P.M.			8 A.M.			5	
	Av. air temperature	Av. soil temperature	Av. per cent humidity	Av. air temperature	Av. soil temperature	Av. per cent humidity	Av. air temperature	Av. soil temperature	Av. per cent humidity	Av. air temperature	Av. soil temperature
(1939)	(°F.)	(°F.)		(°F.)	(°F.)		(°F.)	(°F.)		(°F.)	(°F.)
20 June	...	...	...	...	...	...	...	...	...	...	...
26 June	88·0	92·0	71·8	99·7	97·0	40·3	89·6	89·7	57·1	101·4	94·0
3 July	85·1	90·0	78·1	96·4	94·6	45·3	83·8	88·3	74·4	95·5	92·7
10 July	88·7	94·8	61·4	103·4	100·0	34·8	87·8	92·6	62·1	100·6	98·0
17 July	92·3	96·0	63·8	103·1	101·8	40·6	90·2	93·8	67·6	102·7	98·3
24 July	89·6	89·0	61·8	102·6	99·3	40·1	89·0	90·1	63·8	100·6	93·8
31 July	90·5	95·8	57·7	100·8	100·8	38·9	89·8	92·2	60·9	99·8	96·7
7 Aug.	83·0	91·3	76·6	95·4	95·8	53·9	82·6	86·0	81·0	94·0	90·1
14 Aug.	89·4	92·7	56·3	102·8	100·0	32·1	86·8	88·1	67·8	99·7	95·0
21 Aug.	93·3	96·7	46·7	103·6	101·7	32·2	89·0	87·7	59·8	98·0	91·3
30 Aug.	...	...	...	...	...	...	...	...	...	...	...
4 Sept.	...	...	...	...	...	...	...	...	...	...	...
11 Sept.	...	...	...	...	...	...	...	...	...	...	...
18 Sept.	...	...	...	...	...	...	...	...	...	...	...
25 Sept.	...	...	...	...	...	...	...	...	...	...	...
2 Oct.	...	...	...	...	...	...	...	...	...	...	...

I

and pure cotton plots

only		Cotton+sorghum									
P.M.		8 A.M.			5 P.M.			Per cent mortality			
								Date of removal of sorghum			
Av. per cent humidity	Per cent mortality	Av. air temperature	Av. soil temperature	Av. per cent humidity	Av. air temperature	Av. soil temperature	Av. per cent humidity	20 July	30 July	10 August	16 August
		(°F.)	(°F.)		(°F.)	(°F.)					
...	7·67	...	...	...	...	...	...	0·66	1·66	2·92	0·83
38·7	12·47	87·1	88·8	67·1	97·0	90·6	56·0	1·25	0·83	1·63	0·34
48·8	10·84	83·8	87·0	81·8	91·9	88·5	61·7	0·00	0·08	0·35	0·16
42·1	4·84	85·4	88·3	74·7	98·4	90·1	51·0	0·08	0·00	0·08	0·08
51·2	10·47	88·8	91·0	77·2	101·0	93·4	59·1	0·00*	0·00	0·08	0·00
51·1	12·1	87·7	88·1	71·3	96·4	90·1	60·8	0·00	0·00*	0·00	0·00
41·7	5·72	89·8	89·7	63·6	97·7	93·3	50·4	0·00	0·00	0·00	0·00
61·8	12·47	82·0	85·6	86·1	93·6	89·4	62·7	0·00	0·00	0·00*	0·00
40·8	3·21	85·8	86·3	76·0	96·6	91·0	53·2	0·08	0·00	0·00	0·00
46·8	2·12	87·0	86·0	67·0	98·3	89·0	47·8	2·49	0·08	0·26	0·00
...	1·78	...	...	...	...	...	...	3·35	1·65	0·00	0·00
...	1·2	...	...	...	...	...	...	8·23	3·22	2·14	0·00
...	2·23	...	...	...	...	...	...	6·71	7·02	5·9	0·09
...	0·2	...	...	...	...	...	...	1·68	3·35	4·9	0·8
...	0·2	...	...	...	...	...	...	0·34	0·92	1·2	0·3
...	0·0	...	...	...	...	...	...	0·34	0·23	0·34	0·2

*Stage of removal of sorghum so that the crop was no longer mixed.

It is obvious that close planting of sorghum with cotton will adversely affect the growth of cotton plants. With a view, however, to save the cotton crop from this adverse effect on growth, the sorghum plants must not be left standing in the field longer than is absolutely necessary to reduce the incidence of the disease to the lowest possible limit. In order to find the most advantageous time for the removal of sorghum for this purpose the 16 subplots sown to the mixed crop were divided into four lots of four each and sorghum was removed on four different dates, i.e. on 20 July, 30 July, 10 August and 16 August, respectively.

The experiment was conducted on the randomized system. Counts of mortality due to root-rot were made at weekly intervals both in the mixed and pure cotton plots. Air temperature, soil temperature at 30 cm. depth and humidity records were taken twice a day, i.e. at 8 A.M. and 5 P.M. inside the mixed as well as control plots. Temperature and humidity records were also taken in an uncropped piece of land adjacent to the experimental area. The average per cent mortality from week to week in each set of four plots and temperature and humidity inside mixed and pure cotton plots is shown in Table I. Percentage mortality in each set of plots at the end of the root-rot season before and after removal of sorghum is shown in Table II. Plate LIX fig. 1 shows a plot of cotton soon after removal of sorghum and a check pure cotton plot. Plate LIX, fig. 2, shows control and a mixed plot from which sorghum was removed on 10 August at fruiting stage.

The results recorded in Table I show that :—

1. Mortality in the mixed crop is lower than in pure cotton throughout.
2. About three weeks after removal of sorghum the disease made a fresh start. The highest total mortality occurred in the first lot from which sorghum was removed on 20 July and the least mortality was in the fourth lot from which sorghum was removed on 16 August.
3. Soil temperature is lower in the mixed crop throughout.
4. Air temperature on the whole is lower in the mixed crop.
5. Humidity is higher in the mixed crop.
6. Soil and air temperatures are throughout lower in the cotton than in the un-cropped area.
7. Humidity tends to be higher in the cotton than in un-cropped area.

The data given in Table II bring out the following points of interest :—

1. Mortality before removal of sorghum is appreciably low in all the four groups.
2. Mortality after removal of sorghum is highest in the first group and lowest in the fourth group.
3. The incidence of the disease is reduced to the minimum in the fourth group, i.e. 3 per cent against 69 per cent in control, showing thereby that the best time for removal of sorghum is the middle of August.

It may, however, be mentioned that the plants of the fourth group remained rather small in size and their growth was poor, whereas those of the first and second group almost recovered from the adverse effect of the sorghum crop and their growth proceeded normally afterwards.

(*a*) (*b*)

FIG. 1. (*a*) Plot of cotton soon after removal of sorghum ; (*b*) Check pure cotton plot (Sticks indicate the positions of plants killed)

(*a*) (*b*)

FIG. 2. (*a*) Control pure cotton plot ; (*b*) Mixed plot from which sorghum has been removed on 10 August at fruiting stage

a *b*

FIG. 1. (*a*) Plot of cotton inter-cropped with *moth* (*Phaseolus aconitifolius*); (*b*) Pure cotton plot

FIG. 2. The mixed plot with *moth* removed

TABLE II

Incidence of the disease in relation to the time of removal of sorghum

Set No.	Date of removal of sorghum (1939)	Average per cent mortality		Total per cent mortality
		Before removal of sorghum	After removal of sorghum	
I	20 July . . .	1·99	22·30	23·80
II	30 July . . .	2·50	17·00	18·70
III	10 August . . .	5·00	15·70	20·00
IV	16 August . . .	1·40	1·65	3·40
V	Pure cotton control .	..	..	68·50

B. *Effect of inter-cropping cotton with* moth *and small millets*

The effect of mixed cropping with *moth* (*Phaseolus aconitifolius*), *swank* (*Panicum colonum*) and *kangni* (*Setaria italica*) was tested in a piece of land heavily infected with the disease. *Desi* cotton var. (Mollisoni 39) was sown on 14 May in rows 2½ feet apart. Six rows of cotton were sown in each plot and in between the cotton rows *moth*, *swank* or *kangni* was sown on the same day ; also a border of the same crop 2 ft. in width was sown around the cotton. Three plots were sown with each of the mixtures and three plots were put under pure cotton as checks. The plots were randmomized. Root-rot mortality counts were taken at weekly intervals. Soil temperature, air temperature and humidity were recorded inside the mixed and pure cotton plots at 8 A.M. and 5 P.M. *Moth*, *swank* and *kangni* were removed from cotton plots on 18 August. Results of this experiment are given in Table III. Fig. 1 shows the effect of mixed cropping on the incidence of the disease.

The general conclusions deduced from the data set out in Table III are as follows :—

1. Root-rot mortality in cotton + *moth* is lower than in pure cotton. The differences are highly significant, t being 6·03. For a series of 10 observations a value of t equal to 2·23 is just significant [Fisher, 1925]. Soil temperatures and air temperatures are lower in the mixed crop but humidity is higher than in pure cotton.

2. In cotton + *swank* mortality due to root-rot is lower throughout except in the first week (t = 3·32) but there are no regular differences in soil and air temperatures. Humidity tends to be higher in the mixed crop but the differences are not marked as these vary from —0·2 to +4·7 in the morning and from —2·7 to +3·3 in the evening.

TABLE

Effect of inter-cropping cotton with moth *and*

Week ending	Cotton only							Cotton + *swank* (*Panicum*					
	8 A.M.			5 P.M.				8 A.M.			5 P.M.		
	Av. air temperature	Av. soil temperature	Av. per cent humidity	Av. air temperature	Av. soil temperature	Av. per cent humidity	Per cent mortality	Av. air temperature	Av. soil temperature	Av. per cent humidity.	Av. air temperature	Av. soil temperature	Av. per cent humidity
(1939)	(°F.)	(°F.)		(°F.)	(°F.)			(°F.)	[(°F).		(°F.)	(°F.)	
20 June . .	...	...	...	...	...	...	12·5	...	...	...	...	...	...
26 June . .	89·8	90·4	57·2	99·5	94·5	48·2	6·84	89·6	89·7	58·1	98·7	94·1	48·8
3 July . .	82·3	85·8	80·0	92·6	89·5	55·7	8·19	81·8	86·1	83·3	93·0	89·8	57·8
10 July . .	88·1	90·0	66·4	97·8	95·6	46·4	4·45	88·1	88·7	70·8	99·7	94·4	46·4
17 July . .	89·7	89·5	72·4	95·1	92·6	57·2	11·42	89·7	89·0	76·1	98·1	91·8	57·2
24 July . .	88·0	88·7	66·8	96·6	91·7	57·6	8·15	87·7	88·7	71·5	97·0	92·0	59·6
31 July . .	88·7	91·3	63·8	97·0	94·2	47·7	4·58	89·2	90·2	66·1	98·2	94·5	51·0
7 Aug. . .	82·4	84·6	79·8	90·6	88·3	67·6	9·9	83·0	85·7	83·1	93·0	90·6	64·9
14 Aug. . .	87·3	87·0	62·3	95·1	90·8	50·5	4·0	87·8	87·3	70·3	89·0	92·4	50·1
21 Aug. . .	88·3	87·0	59·5	94·7	88·7	51·0	1·73	90·3	88·0	59·3	97·7	92·0	51·8

III

small millets on the incidence of root-rot disease

Colonum	Cotton + *kangni* (*Setaria italica*)							Cotton + *moth* (*Phaseolus aconitifolius*)						
Per cent mortality	8 A.M.			5 P.M.			Per cent mortality	8 A.M.			5 P.M.			Per cent mortality
	Av. air temperature	Av. soil temperature	Av. per cent humidity	Av. air temperature	Av. soil temperature	Av. percent humidity		Av. air temperature	Av. soil temperature	Av. per cent humidity	Av. air temperature	Av. soil temperature	Av. per cent humidity	
	(°F.)	(°F.)		(°F.)	(°F.)			(°F.)	(°F.)		(°F.)	(°F.)		
13·66	...	...	...	...	...	...	11·0	...	...	...	...	...	...	5·52
6·56	89·7	89·8	69·3	95·8	93·7	49·4	11·5	89·2	91·0	66·6	98·8	94·1	45·9	4·43
6·81	82·1	86·1	82·4	91·3	88·3	63·6	15·35	80·1	84·3	85·7	89·4	86·3	68·3	1·48
1·77	85·6	88·0	73·0	96·3	91·0	54·8	3·87	84·3	84·3	82·2	93·0	86·4	63·4	0·00
0·45	88·7	87·7	79·0	94·8	90·1	65·2	0·26	85·8	84·8	85·1	92·3	87·1	71·1	0·00
0·00	87·6	87·4	73·8	94·8	89·8	64·6	0·27	86·0	85·3	82·1	92·6	87·6	69·3	0·00
0·22	88·5	89·2	66·4	97·2	92·3	51·6	0·00	86·0	86·0	77·7	91·8	88·5	64·4	0·00
0·00	82·8	85·3	83·3	91·6	88·6	71·6	0·00	81·0	63·3	88·8	89·0	86·1	79·7	0·00
0·00	87·7	87·1	68·8	93·3	91·4	53·4	0·00	85·3	84·7	77·1	91·8	88·0	69·5	0·00
...	89·7	88·0	62·1	96·0	90·7	56·7	0·00	86·0	84·7	76·8	91·3	87·3	67·2	0·00

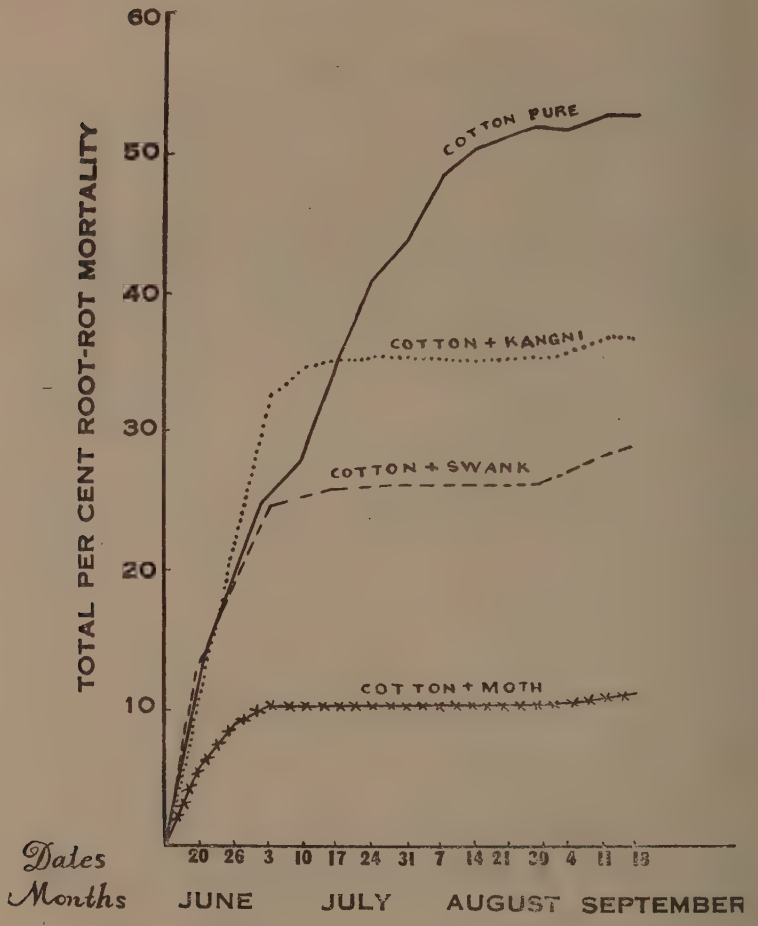

FIG. 1. Effect of mixed cropping on root-rot mortality

3. In cotton + *kangni* root-rot mortality is high in the first three weeks but after 3 July 1939 there is a marked decline in the death rate. *Kangni* plants were very small in size during the first few weeks and did not serve as a cover to cotton plants. The differences in mortality are, however, insignificant, t being 1·58. There are no regular differences in soil and air temperatures but humidity is higher in the mixed crop throughout. The differences in the mornings vary from 2·4 to 12·1 and 1·2 to 8·4 in the evenings.

It might, however, be mentioned that germination and growth of *swank* and *kangni* were very poor. Germination was delayed on account of dryness of the weather. Both the crops therefore did not materially help in reducing soil and air temperatures in the mixed crop. All the same, it is indicated that *swank* and *kangni* tend to reduce root-rot mortality though the results are

not significant in the case of the latter crop. It is probable that if the crop had grown normally, the incidence of the disease would have been further reduced in both the cases.

Where *moth* was sown in between the cotton rows, root-rot mortality was observed only during the first couple of weeks in the spots where *moth* had not yet grown properly. *Moth* neither shaded nor greatly affected the growth of cotton plants. Plate LX, fig. 1 shows a plot of cotton intercropped with *moth* and a pure cotton check plot. Plate LX, fig. 2 shows the mixed plot with *moth* removed.

An experiment was conducted in 1940-41 in order to find the most suitable time for the removal of *moth*. *Desi* cotton variety Mollisoni 39 was sown in 20 heavily diseased plots on 16 May 1940. *Moth* was inter-cropped in 16 plots. The remaining four pure cotton plots served as controls. The experiment was conducted on the randomized system. Mortality, temperature and humidity records were taken as usual. *Moth* was removed from four plots at a time on different dates. The results of the experiment are summed up in Table IV.

TABLE IV

Incidence of the disease in relation to the time of removal of moth

Set No.	Date of removal of *moth* (1940)	Average per cent mortality		Total per cent mortality	Yield per acre	
		Before removal of *moth*	After removal of *moth*		Green *moth* Md. Sr. Ch.	Seed cotton Md. Sr. Ch.
I	1 Aug. .	0·86	0·00	0·86	209 22 0	12 6 12
II	15 Aug. .	0·67	0·00	0·67	202 5 0	10 20 12
III	30 Aug. .	0·45	0·00	0·45	167 19 0	8 30 10
IV	22 Oct. .	1·1	0·00	1·1	74 34 12	7 6 11
V	Pure cotton	..	..	52·51	..	10 16 10

The results show that total mortality in the mixed plots is almost negligible, i.e. about 0·5-1 per cent as against 53 per cent in the pure cotton plots. They also show that after the removal of *moth* on different dates no deaths occurred due to root-rot, indicating thereby that in order to reduce mortality to an appreciable degree it is quite safe to remove *moth* as early as the first of August.

Yield of green *moth* in different cuttings varied from 209½ mds. to 75 mds. per acre. Yield was much reduced in the last cutting because the fodder had partly dried up. Yield of seed cotton in the first lot tends to be higher than pure cotton control plots, but the difference is not significant. The yield from the second lot is almost the same as in the controls, whereas the yield

of the second and third lots is lower than the controls. It might, however, be mentioned that soil and air temperatures were lower in the mixed crop, whereas humidity was higher throughout.

At Lyallpur, in addition to *moth*, cowpea (*Vigna catiang*), *guara* (*Cyamopsis psoralioides*) and sorghum were also sown mixed with *desi* cotton variety Mollisoni 39 to test their effect on the incidence of the disease. The sowings were done on 17 May 1940 and the crops were removed on the 16 August. The results of the experiment are given in Table. V.

TABLE V

Effect of inter-cropping desi *cotton with different crops*

Treatment	Average per cent mortality (3 plots)	Yield per acre					
		Green inter-crop			Seed cotton		
		Md.	Sr.	Ch.	Md.	Sr.	Ch.
Cotton + cowpea . . .	10·05	311	15	11	7	24	10
Cotton + *guara*	2·40	588	23	2	6	33	7
Cotton + sorghum . . .	0·87	357	6	10	3	29	1
Pure cotton	50·89	..			10	21	5

Plants of all the crops inter-cropped in between the cotton rows grew tall and shaded the cotton plants, resulting in their poor growth and reduced yield. Soil and air temperatures were lower in the mixed crop, whereas humidity was higher than in the pure cotton check plots.

Four more experiments were carried out in 1940-41, both at Lyallpur and B. C. G. A. Farm, Khanewal, to test the effect of mixed cropping with *moth* as a measure to control the disease.

In two experiments American cotton (*G. hirsutum*) was inter-cropped with *moth*. One experiment was laid out at Lyallpur and the other at Khanewal. Another set of similar experiments was laid out in which *desi* cotton (*G. indicum*) was intercropped with *moth*.

All these experiments were conducted on randomized system in heavily infected plots and the sowings were done in the middle of May. Soil and air temperatures and humidity records were taken only at Lyallpur. These confirmed the previous findings that the temperature is lower in the mixed plots, whereas humidity is higher.

The results of all the four experiments are summed up in Table VI. Fig. 2 shows the effect of inter-cropping American cotton with *moth* on the incidence of the disease (var. LSS).

TABLE VI

Effect of inter-cropping desi *and American cottons with* moth *on the incidence of the disease and yield of seed cotton*

Station	Treatment	Variety	Av. per cent mortality	Av. yield per acre	
				Green *moth*	Seed cotton
				Md. Sr. Ch.	Md. Sr. Ch.
Lyallpur .	American cotton + *moth*	LSS .	0·92	190 6 15	15 15 9
	Pure cotton (control)	LSS .	63·09	..	4 25 2
	Difference of means per plot				5·37 Sr.
	S. E. of the difference per plot				0·47
	t				11·40
Khanewal .	American cotton + *moth*	KT 25	2·98	220 35 11	12 24 5
	Pure cotton (control)	KT 25	45·92	..	4 13 0
	Difference of means per plot				11·20 Sr.
	S.E. of the difference per plot				0·92
	t				12·20
Lyallpur .	*Desi* cotton+*moth* .	Mollisoni 39	2·42	222 23 2	14 25 8
	Pure cotton (Control)	Mollisoni 39	55·35	..	11 5 6
	Difference of means per plot				1·74 Sr.
	S.E. of the difference per plot				1·56
	t				1·12
Khanewal .	*Desi* cotton+*moth* .	Mollisoni 39	1·81	220 35 11	15 33 3
	Pure cotton (control)	Mollisoni 39	52·22	..	9 8 2
	Difference of means per plot				9·00 Sr.
	S.E. of the difference per plot				0·71
	t				12·67

The results show that root-rot mortality is markedly reduced in the mixed crop and also yield of American seed cotton is higher in the mixed plots both at Lyallpur and Khanewal than in the pure cotton check plots. The differences are highly significant.

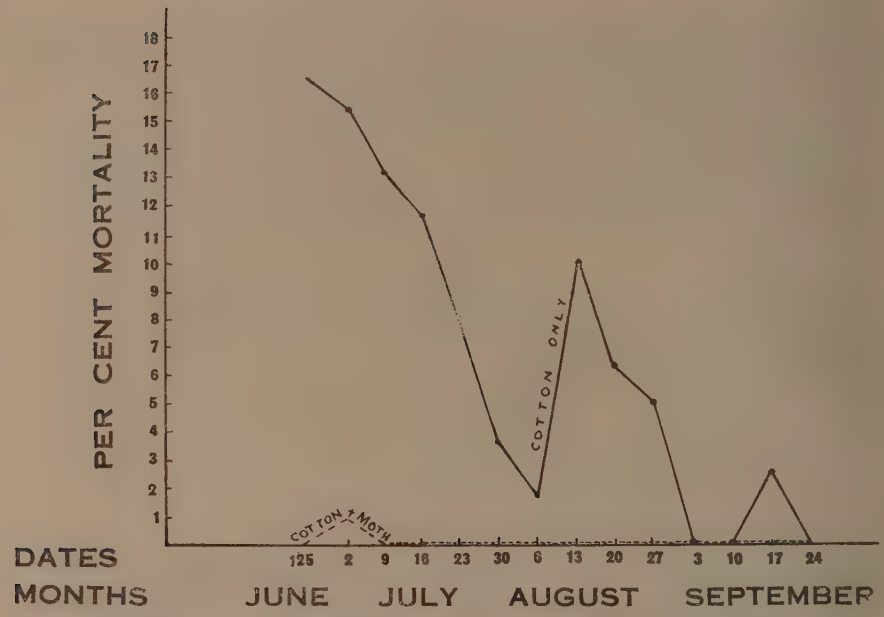

FIG 2. Effect of inter-cropping cotton with *moth* on the incidence of the disease (var. LSS), Lyallpur, 1940-41

In the case of *desi* cotton the yield is higher in the mixed plots both at Khanewal and at Lyallpur, but the results are significant only in the former case. It appears that the growth of plants and the yield of seed cotton is adversely affected in the case of *Desi* cotton when it is sown mixed with *moth*. Sufficient data are not yet available to explain this difference between *desi* and American cottons. This may either be due to the habit of the plant, or to the fact that American cotton gets sufficient time to make good the loss due to inter-cropping as it matures about 1½ months later than the *desi* cotton.

Later on it may be made possible by certain modifications, such as adjustment of sowing time or time of removal of inter-crop to get remunerative returns even from *desi* cottons. It should be remembered that an additional income accrues from the fodder crop raised from the mixed plots.

Further work is required to investigate the causes of reduction in mortality in cotton sown in mixture with various crops. Temperature may be an important factor, but it is likely that some other factor also comes into play which helps in reducing the incidence of the disease, as in the case of *swank* where the temperature was not materially affected in the mixed crop but the incidence of the disease was reduced.

SUMMARY

1. When cotton is inter-cropped with sorghum or *moth* (*Phaseolus aconitifolius*) the incidence of the root-rot disease is significantly reduced. Soil and air temperatures are lower within the mixed crop but humidity is higher than in the pure cotton plots.

2. Two varieties of American cottons when sown in mixture with *moth* gave higher yields than the pure cotton.

3. Incidence of the disease is also reduced when cotton is sown in mixture with certain other crops.

Acknowledgements

The author is greatly indebted to Mr H. R. Stewart, C.I.E., I.A.S., Director of Agriculture, Punjab, and to Professor Rai Bahadur Jai Chand Luthra, I.A.S. for their keen interest and helpful suggestions throughout the course of this investigation.

Thanks are also due to Dr. W. Burns, C.I.E., I.A.S., Agricultural Commissioner with the Government of India, for his valuable suggestions and criticism. Assistance given by Mr Mulk Raj Sikka, Research Assistant and Mr Hans Raj Sikka, Fieldman, is gratefully acknowledged. The funds for the investigation were provided by the Indian Central Cotton Committee.

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PINK DISEASE OF ORANGE TREES IN THE CENTRAL PROVINCES

BY

JEHANGIR FARDUNJI DASTUR, M. Sc., D. I. C.

Mycologist to Government, C. P. and Berar, Nagpur

(Received for publication on 12 April 1941)

(With Plates LXI–LXIII)

PINK disease caused by *Corticium salmonicolor* B. & Br. is widespread both as regards its geographical distribution and its range of hosts. In the Central Provinces, however, this disease causes serious damage only to one host, viz. orange trees (*Citrus aurantium*) and that too only in a restricted locality. Orange cultivation is spread over several districts in this province but so far the disease has been known to occur only in Balaghat, Betul, Jubbulpore and Ramtek (Nagpur district). In Jubbulpore and Ramtek only stray cases of infection have been recorded. In Betul this disease in 1939 was found on a number of orange trees but last year the disease was not reported to have done any further damage. In Balaghat the disease is usually in an epidemic form and is a serious threat to the further expansion of orange orchards in this district.

Symptoms

The disease does most damage during the wet season. The presence of the disease is readily noticed when the leaves of a branch wilt, turn yellow and drop. If the diseased branch is examined, the cause of its dying from the top and of the defoliation or wilting of the leaves is evident ; the affected part of the branch is either covered with a fine silvery white film named ' spinnengewebe ' by Rant [1911] or is studded with white or pink coloured raised pustules of the size of a pin head, or it is covered with pinkish coloured pockmarks caused by the flaking off of scales of the bark giving this part of the branch a general pinkish appearance. In the case of thin branches and twigs the bark when badly diseased is in shreds and the wood is exposed. In some cases the infection can only be detected by the presence of cankers on the twigs and small branches (Plate LXI, fig. 1).

The secondary effects of the disease on a branch are (1) shredding of the bark, (2) scaling or exfoliating of the bark, and (3) gumming and development of cankers.

As a result of the development of pustules the bark becomes dry, the thin-walled cells are destroyed, and the bark is torn into ribbons or fibrous shreds ; this happens especially in the case of infected branches which are not thicker than one's finger. Usually this tearing up of the bark is not associated with the presence of the spinnengewebe hyphae.

In some cases small thin pieces of bark of the branches exfoliate. The result is that small shallow depressions or pits or pock marks are formed on the surface ; when fresh they are distinctly pink or pale rose coloured, and

1 2

FIG. 1. An orange twig showing cankers formed by *Corticium salmonicolor*

FIG. 2. An orange branch showing the spinnengewebe mycelium

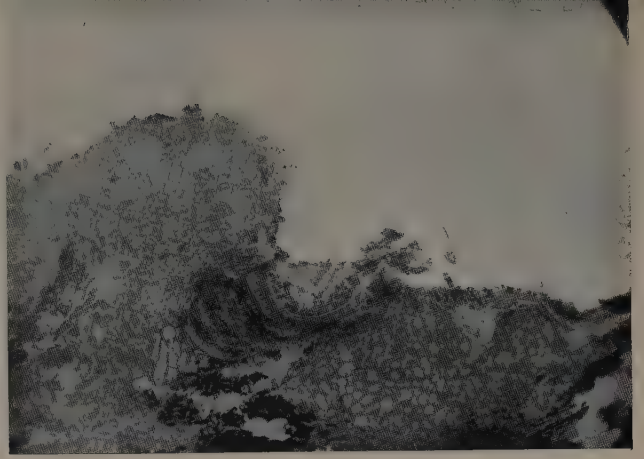

FIG. 3. A photo-micrograph of a deep seated pustule bursting through the bark. Note the presence of host cells in the pustule

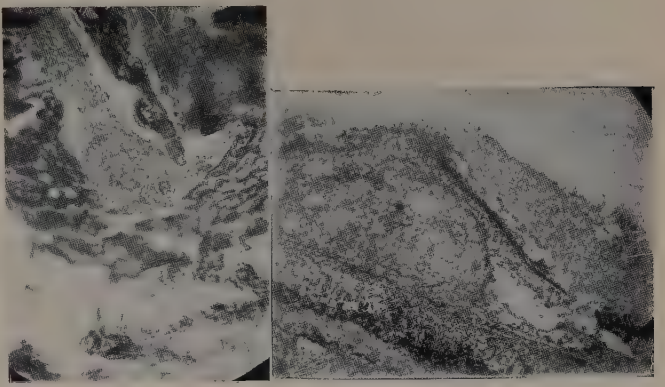

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FIG. 4. A photo-micrograph of a stromatic aggregate of cells formed in a fold of the bark

FIG. 5. A photo-micrograph of a pustule developed underneath

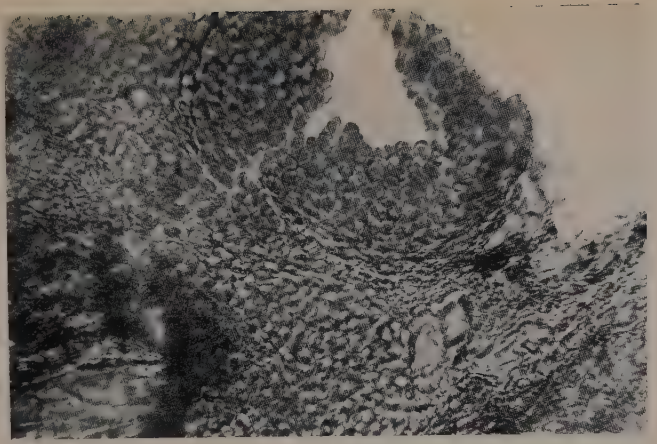

FIG. 1. A photo-micrograph of a *Necator* pustule with spores

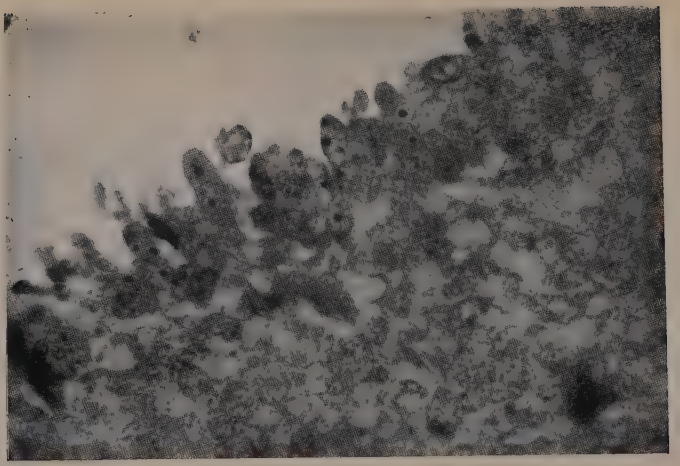

FIG. 2. A photo-micrograph of a part of the basidial stage, showing basidia formed in a row from the hymenial layer

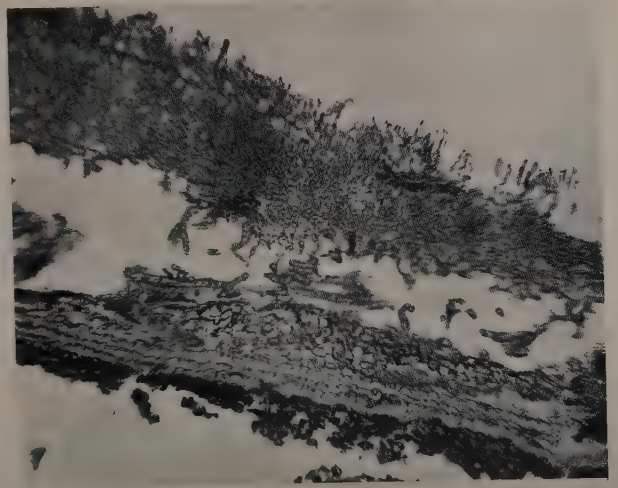

FIG. 3. A photo-micrograph showing a section of the basidial stage ; the basidia are scattered

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FIG. 4. A semi-diagrammatic drawing of a section through a pit in the bark showing the remnants of a pustule over the host tissues (× 65)

FIG. 5. An enlarged view of a part of the above section (× 600)

when they are numerous the affected limb has distinctly a general pink coloured appearance. With age the colour of the pits changes to dirty white or pale brown. The exfoliating of the bark may at times be accompanied with slight exudation of gum. The scaling of the bark takes place where pustules had developed. If sections through the pits are examined under the microscope, a layer of pseudoparenchymatous fungus tissue or scattered, short, roundish or angular fungus cells are readily seen ; they are the basal remnants of the pustules which have dropped off with the exfoliating bark (Plate LXII, figs. 4, 5).

Occasionally, vertical small cracks are formed on the diseased bark from which drops of gum exude. When the development of gum pockets is confined to tissues outside the cambium, new meristematic tissue is developed below the gum pocket which later is scaled off. But when the gum pocket destroys the cambium the bark above the affected part sloughs off, exposing the discoloured wood ; a canker is formed, which may be a large gaping wound or may be very small and not readily noticeable. In the tissues of the callus formed round the wound are sometimes seen inter-cellular strands of hyphae or small aggregates of fungus cells resembling a stroma. These inter-cellular hyphae may very probably be the dormant mycelium of *Corticium salmonicolor* ; the stromatic aggregates are similar to the stroma of the pustules described above.

Description of the fungus

The spinnengewebe form is at first silvery white in colour with feathery or cottony margins ; as the film extends up and down the limb, zones of feathery margins are distinctly visible (Plate LXI, fig. 2). This is specially noticeable when the affected limb is thick ; when the diseased twig is thin the margins are not conspicuously feathery. At a later stage the silvery white colour of the film changes to a general pink colour, except at the margins which are still feathery and white. With age the spinnengewebe film becomes dirty drab coloured. In the dry weather there are no externally visible signs of this film ; but under the microscope remnants of the hyphae can be seen in the crevices and folds of the bark as roundish or angular short cells.

The spinnengewebe form at first consists of a smooth mycelial felt on which at a later stage are seen white cushion-like growths or pustules, which are scattered or in linear rows. This mycelial felt is composed of long strands of hyaline thin-walled, sparsely-septate hyphae, 7-15 μ broad, running parallel to each other ; their branches interlace together ; no clamp connections have been observed. These hyphae grow chiefly superficially on the bark ; they are seen to enter the bark tissues only through a crack in the bark (Plate LXIII, fig. 9), or through the thin-walled cells of a lenticel. When hyphae are over a lenticel or the broken tissues of the bark they lose their filmy character and form a loose aggregate of thin-walled short cells (Plate LXIII, fig. 11).

The pustules or cushion-like growths are white or pink or orange-red or rose coloured. The white-coloured pustules are either wholly superficial on the bark or partly within the bark tissues ; but the pink or rose or orange-red coloured pustules are always wholly or partly embedded in the host tissues.

The white cushions are usually associated with the cobwebby or the spinnengewebe mycelium (Plate LXIII, fig. 2). The hyphae on the surface of the bark loosely aggregate together and form white cushion-like structures. The hyphae remain thin-walled, and the contents are neither granular nor different from those of the spinnengewebe mycelium, but the hyphae forming these cushions are distinctly narrower than those of the spinnengewebe form. These cushions are identical with the sterile white bodies described by Zimmermann [1901].

There is another type of white pustules. The hyphae of the cobwebby or spinnengewebe mycelium over a lenticel or over broken tissues of the bark or in a fold of the bark break up into short roundish or angular cells and form an aggregate of cells resembling a pseudoparenchymatous tissue (Plate LXI, fig. 4). These pustules may remain superficial or they may develop hyphae which may penetrate the host tissues where they are broken, or through the thin-walled cells of lenticels. At first the hyphae are intercellular ; the cell-walls of the host cells soon collapse under the pressure of the fungus tissue and the hyphae then become also intra-cellular. The hyphae from these pustules occasionally form aggregates under the epidermis or in the bark tissues or in the cortex (Plate LXIII, figs. 1 and 2).

The origin of the pink or orange-red pustules seems to be different from that of the white pustules described above. The hyphae or the pseudoparenchymatous aggregates inside the host tissues may lie dormant for a time, e.g. during the dry season, and under favourable conditions resume their growth and burst through the living tissues of the host.

The pustular forms of *Corticium salmonicolor* have been named by Rant [1910 and 1911] as Höckerchen form and ' Knobbeltjesize ' form. According to him Höckerchen are the white pustules formed on the bark by a collection of thin-walled hyphae mostly on lenticels ; Zimmermann [1901] has also described these bodies though he has given no specific name to them ; they are superficially developed, white, round bodies consisting of thin-walled cells the contents of which are poor and therefore he does not consider them to be similar to sclerotia. According to Brooks and Sharples [1914] ' Pink disease frequently assumes the form of white or pale pink pustules arranged more or less in lines parallel with the branches ; this is the Höckerchen form of Rant'. Butler [1918] calls the pustular stage the nodular form which is white and which occurs chiefly in the lenticels. To Lee [1919] the sterile dirty white to pinkish coloured pustules ' which push through the hardened bark ' of *Citrus* ' appear to be the Höckerchen form described by Rant'. Subba Rao's description [1936] of the Höckerchen form is confused. It develops by some of the hyphae of the cobwebby mycelium aggregating ' here and there on the surface of the branch into small cushions of a pinkish or whitish colour' ; he further states that the hyphae form similar condensations or aggregates beneath the layer of cortical cells . His illustration No. 4, which is similar to our microphotograph (Plate LXI, fig. 3), cited by him in support of this description does not bear out his statement ; but this illustration (in the ' Explanation of Plates ') is correctly described by him as ' the pustular masses bursting through the tissues '. He describes the development of the pustules as superficial, whereas he illustrates the development as deep seated in the tissues of the host. Subba Rao has observed but failed to

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PLATE LXIII.

1. A section of the bark showing an aggregate of cells formed in the sub-epidermal cells (× 600); 2. A section of the bark showing aggregates of cells inside and outside the bark (× 600); 3. A deep seated pustule bursting through the bark (× 130) (Note the presence of host cells in the erumpent part of the pustule and the columner sub-epidermal cells); 4. A dormant Necator pustule underneath the bark (× 600); 5. A Necator pustule over a lenticel (× 130); 6. Basidia (× 600); 7. Section of the bark with the basidial stage. Note the different layers of the basidia and the collapse of some of the hymenial cells forming a sort of a thick protective layer (× 600); 8. *Necator* spores. Some of the spores are germinating. 9. A hypha of the spinnengewebe mycelium entering the bark through a crack (× 600); 10. A section of the living bark showing the presence of *Diploidia* sp. and of *C. salmonicolor*: *a 1*, a pycnidium of *Diploidia* sp., *a 2*, *Diploidia* mycelium; *b*, scattered cells of *C. salmonicolor* (× 130); 11. A loose aggregate of cells formed by the hyphæ of spinnengewebe mycelium in a crevice in the bark (× 600); 12. A section through the living bark of a fork showing dormant cells of *C. salmonicolor* (× 600)

PLATE LXIII.

1. A section of the bark showing an aggregate of cells formed in the sub-epidermal cells (× 600); 2. A section of the bark showing aggregates of cells inside and outside the bark (× 600); 3. A deep seated pustule bursting through the bark (× 130) (Note the presence of host cells in the ruptured part of the pustule and the columner sub-epidermal cells); 4. A dormant Zecator pustule underneath the bark (× 600); 5. A Zecator pustule over a lenticel (× 130); 6. Basidia (× 600); 7. Section of the bark with the basidial stage. Note the different layers of the basidia and the collapse of some of the hymenial cells forming a sort of a thick protective layer (× 600); 8. Zecator aporse. Some of the spores are germinating. 9. A hypha of the spinemyewebe mycelium entering the bark through a crack (× 600); 10. A section of the living bark showing the presence of Diplodia sp. and of C. sulmonicolor: a 1, a pycnidium of Diplodia sp., a 2, Diplodia mycelium; b. scattered cells of C. sulmonicolor (× 130); 11. A loose aggregate of cells formed by the hyphe of spinemyewebe mycelium in a crevice in the bark (× 600); 12. A section through the living bark of a lork showing dormant cells of C. sulmonicolor (× 600)

distinguish between the two types of pustules, that which originates on the bark from the spinnengewebe mycelium and that which develops from within the host tissues.

Microtomic and hand sections of the orange bark having these deep seated pustules clearly show that they consist of both fungus and host cells (Plate LXI, figs. 3 and 5 ; Plate LXIII, fig. 3). When the dormant mycelium in the cortex of the host tissue becomes active, there is an abnormal development of the host cells and a compact globular body with a fringe of hyphal strands, the immature pustule is developed beneath the brown epidermis or the phellem ; under pressure of the growth of the fungus this globular body bursts through the bark tissues forming an erumpent pustular outgrowth. In the part of the pustule which is outside the bark tissues the host cells are embedded in or surrounded by the pseudoparenchymatous fungus tissue, are thin-walled and wholly empty ; their lumen is much bigger than that of the fungus cells ; remnants of collapsed host cells may also be readily seen ; but in the lower part of the pustule underneath the ruptured epidermis the host cells lose their uniform shape and become paliform ; they are wholly or partly filled with fungus cells which are usually pseudoparenchymatous, but occasionally are also fibrilar (Plate LXIII, fig. 3). When sections are stained with Cotton Blue and Saffranin the host cells in the pustules are stained red and the fungus cells blue. Subba Rao's illustration distinctly shows the pustules composed of host and fungus cells (Compare his illustration No. 4 with our Plate LXI, fig. 3 and Plate LXIII, fig. 3). At the base of the pustule in the cortex or phelloderm is an aggregate of closely septate fibrillate hyphae which has completely destroyed the host cells ; from this mycelial aggregate, which is not pseudoparenchymatous, hyphae spread out fan-like in the neighbouring cells except the stone cells.

There is a third type of the pustular stage, known as the *Necator* stage. When the spores are developed it can be readily distinguished from the other two types of pustules by its orange-red colour. This is the *Necator decretus* form of the fungus. The pustules are usually formed in vertical elongated streaks, as the pustules are developed on lenticels or in the tissues covered by the vertically elongated, slightly depressed green streaks which, as described by Webber and Fawcett [1935], are normally found in the grayish or brownish bark of an orange tree five to six years old.

The *Necator* pustule when developed over a lenticel is superficial ; it is generally seated on the narrow band of suberized cells (Plate LXIII, fig. 5). As observed by Rant [1911], it is also developed underneath the epidermis or in the periderm ; a number of these pustules may run together and are separated from each other by a partition wall formed by the host tissues. Even when spores have not been formed, sections of *Necator* pustules under the microscope can be readily distinguished from those of the sterile deep-seated pustules described above. The healthy tissues of the host are sharply delimited from the tissues destroyed by the *Necator* pustules ; from the base of these pustules there are no ramifications of the mycelial threads into the healthy tissues ; in the case of the other type of deep-seated pustules the hyphae are found to have penetrated the host tissues beyond the mycelial aggregates.

The *Necator* pustule has a superficial resemblence to a pycnidium, especially when it is full of the waxy orange-red mass of irregularly shaped

spores ; but in its development it is different from a pycnidium. Brooks and Sharples correctly describe the development of the *Necator* pustule. Underneath the cuticle or in the periderm of the host branch there is an aggregate of fungus tissue forming a pseudoparenchymatous stroma (Plate LXIII, fig. 4) ; under favourable humid conditions this stromatic structure may burst through the bark and become erumpent ; the component cells of this stromatic tissue break up into individual cells, each cell being a spore (Plate LXII, fig. 1 ; Plate LXIII, fig. 5). As pointed out by Brooks and Sharples it is because of this mode of development of the spores that they are so irregular in shape and size (Plate LXIII, fig. 8). Under dry conditions the stromatic bodies originating underneath the bark tissues may remain dormant and embedded underneath the cuticle or the suberized or lignified tissues of the bark (Plate LXIII, fig. 4) ; or they may partly burst through the bark and remain partially covered by the ruptured cuticle of the layer of suberized cells ; they do not develop further and remain as pink coloured sterile pustules or cushions ; but under humid conditions they turn orange red in colour and this change of colour synchronizes with the breaking up of the pseudoparenchymatous tissue into numerous one-celled, irregularly-shaped bodies of spores.

The pustule formed over a lenticel is developed similarly. The hyphae form a pseudoparenchyma over the outer suberized layer of the lenticel ; the component hyphae do not extend beyond the pseudoparenchymatous tissue of the pustule. This pustule is in every way identical with that formed underneath the epidermis except that it is superficial and single.

Subba Rao [1936] has described the *Necator* pustules on tea branches to be ' composed of a plectenchyma which is tuberiform with a finer and resistant pseudoparenchymatous cortex and a looser, less homogeneous prosenchymatous core '. The *Necator* pustules on the orange branch have not been observed to have these two types of tissues which can be differentiated into a core and a cortex. The pustule is homogeneous in structure and the spores are developed by the component cells of the pseudoparenchymatous tissue of the pustules breaking up into individual unseptate cells as described above. They have never been found to ' originate by a process of abstriction from the paraplectenchymatous cells of the cortex ' as described by Subba Rao (Plate LXII, fig. 1 ; Plate LXIII, fig. 5).

The necator spores collectively are pink in colour but individually they are colourless. They are thin-walled ; they vary considerably in shape and size ; they may be angular or roundish, long or short ; they measure 8—20 × 5—10 μ. They germinate readily in water.

On rare occasions, there are on the *Citrus* bark, crust-like patches of fungus growth. This growth spreads over the surface of the bark, covering a very small and limited area, and is conspicuous when fresh by its bright pink or almost orange-red colour ; with age this growth becomes dull salmon coloured. It is different in appearance to the spinnengewebe or Höckerchen or other forms of this fungus. The margin of this growth is determinate but irregular in outline. The surface is not shiny as is that of spinnengewebe form and is not smooth. The surface is very much like that of a patch of coloured lime that has dried on the bark and has cracked. This crust-like growth is very much appressed to the bark ; when closely examined it is seen to have formed

a sort of an irregular reticulum through the openings of which minute parts of the bark are distinctly visible as brown or black islands surrounded by strands of fungus mycelium. This is due to the incomplete development of the fungus over this area.

In sections the crust may be over 5-30 μ thick ; it is composed of a loose subiculum formed of long strands of hyphae ; these hyphae are broad and sparsely septate ; the walls of the hyphae may be encrusted. From this subiculum arises a broad layer of reticulated or pseudoparenchymatous cells (Plate LXII, fig. 3). In some cases the upper cells of this reticulated layer collapse, forming a thickish layer resembling the cuticular or suberized or thickened layer of an epidermis (Plate LXII, fig. 3 ; Plate LXIII, fig. 7); in other cases the upper cells of the reticulated layer develop the hymenial layer from which arise broad unseptate club-shaped bodies, the basidia, and septate, branched or unbranched broad hypha-like bodies with rounded apices, the paraphyses (Plate LXIII, fig. 6). The basidia measure $16 \cdot 6—33 \cdot 2 \times 5—8$ μ. From the hymenial layer the basidia may be developed in rows (Plate LXII, fig. 2 ; Plate LXIII, fig. 7) as suggested by Zimmermann's description [1904] and figures or the basidia may be scattered and irregularly arranged (Plate LXII, fig. 3) as observed by Brooks and Sharples.

Cystidia have not been observed. The basidia are plurinucleate, but sterile (Plate LXII, figs. 2 and 3 ; Pate LXIII, figs. 6 and 7) ; they bear neither sterigmata nor basidiospores. This hymenial layer with basidia and paraphyses is more deeply stained with Haematoxylon or Cotton Blue than the other layers of this form.

Dissemination of the fungus

Corticium salmonicolor on orange trees in this province is in an active state only during the wet season, when, as we have seen, it develops various forms, viz. the sterile pustular forms, the *Necator* form, the basidial form and the spinengewebe form. All these forms are capable of spreading the disease during the wet season to healthy plants or to healthy limbs of the same plant ; the infective material can be carried directly or on the scales of the exfoliating bark by rain-water, insects or wind. But all of these forms cannot play an important part in the annual incidence of the disease. The spinnengewebe form is short-lived and not capable of over-wintering or over-summering ; the mycelium is thin-walled and does not contain reserve food material ; even the white pustules which are developed superficially from this mycelium are equally seasonal in growth ; with the beginning of the dry season these white pustules and the spinnengewebe mycelium shrivel up. The basidial form, being rare and sterile, is equally unsuitable for the perpetuation of the parasite from one wet season to another. The *Necator* form that has developed spores would also be capable of disseminating the fungus only during the wet season as the spores are thin-walled, unless their waxy covering protects them through the long dry period ; but the *Necator* pustules with their stromatic tissues not broken up into spores would be eminently suitable to withstand dry weather and would be resistant to adverse conditions, especially those that are underneath the bark or the thickened epidermis. The sterile pustules and aggregates of fungus cells which are developed in the tissues of the bark

would also serve as potential sources of infection when the dry period is succeeded by the wet season, especially those which are dormant in the living tissues of the bark.

The possibility of the strands of mycelium found in the callus tissues round cankers or in the tissues of lenticles remaining dormant cannot be overlooked. It is not improbable that these isolated strands of hyphae over-winter and over-summer in the plant tissue and resume their growth in the rainy season. Field observations do not overrule this source of infection. In some cases, in the rainy season, in absence of any of the other forms of the *Corticium* fungus, a few isolated and scattered white sterile pustules bursting through lenticles or the bark round cankers or in the fork have been observed ; it is not improbable that they may have originated from the dormant mycelium inside the bark.

Brooks and Sharples [1914] have recorded that a species of *Nectria* is often found along with *Corticium salmonicolor* ; on orange branches also these two fungi are associated together, especially when the limb affected by the pink disease is thick. The perithecia of *Nectria* sp. have been found to be superficial and seem to grow on the dead outer tissues of the bark. There is another fungus which is more intimately associated with *C. salmonicolor*, and that is *Diplodia* sp. (Plate LXIII, fig. 10). The growth of this *Diplodia* is both external and internal ; the mycelium travels inside the cortex ; stromatic masses are formed in the bark tissues, and pycnidia burst through the epidermis or phellogen, as do the pustules of *C. salmonicolor*. The hyphae and stromatic masses of *Diplodia* sp. can be readily distinguished from those of *C. salmonicolor*. The fungus tissue of *Diplodia* is brown or honey coloured, thick walled ; the cell contents are not readily visible because of the dark-coloured walls. The fungus tissue of *C. salmonicolor* is hyaline and thin-walled, and the cell contents are granular. The aggregates of fungus cells of *C. salmonicolor* are often embedded in or surrounded by the stromatic tissues of *Diplodia* in the host tissues. It is not improbable that the thick-walled stromatic masses of *Diplodia* sp. in the outer or inner tissues of the bark serve as a protective covering to the scattered thin-walled aggregates of the pink disease fungus and enable them to tide over the dry season. It has been mentioned above that the hyphae from the spinnengewebe mycelium enter the bark tissues through lenticels or through cracks in the bark ; it is not improbable that the primary infection of the host tissues may also be through the outer cells of the bark killed or weakened by *Diplodia* sp.

Contributing conditions

In Balaghat district, citrus orchards are only a few in number and yet the incidence of pink disease is annual and usually in an epidemic form ; whereas in other parts of the Central Provinces, e.g. Nagpur district, there are extensive areas under citrus but the disease is generally absent, or where found, it is sporadic and confined only to a few isolated trees. In Balaghat the average rainfall from 1 June to 31 October is 60·83 in.. and the average number of rainy days for the same period is 63·1 ; whereas in Nagpur, Jubbulpore and Betul for the same period the average rainfall is 44·79, 51·28 and 40·06 in. respectively, and the average number of rainy days is 54·0, 55·5 and 51·3

respectively. The high average rainfall and the larger average number of rainy days in Balaghat may account for the presence of the disease in an epidemic form. Our inoculation experiments at Nagpur have shown that the inoculated plants are not a source of infection to healthy citrus plants in the same plant house ; and that the infection from the successfully inoculated twig does not spread to the healthy limbs of the same plant the following wet season. This shows that climatic conditions at Nagpur at least are not suitable for the spread of this disease.

Inoculation experiments

Inoculations of potted orange (*Citrus aurantium*) and sour lime (*C. acid* plants, two to three years old, with pure cultures of *Corticium salmonicolor* isolated from orange and mango plants attacked by this fungus were carried out at Nagpur. The inoculum was cultivated aseptically on sterilized small pieces of orange twigs ; they were tied to the stems of the plants to be inoculated ; in some cases water-soaked cotton wool was wrapped round the stem and the inoculum. The inoculated part of the stem was enclosed in a glass or celluloid chimney the open ends of which were closed with wet cotton wool. The inoculations were successful. At first the spinnengewebe form of the fungus developed on the inoculated limb ; later, white pustules were formed along with the spinnengewebe mycelium ; small drops of gum were seen exuding from very thin small cracks in the bark covered by this mycelium ; the leaves of the inoculated twig turned yellow and wilted ; with the further progress of the inoculation the part of the twig above the inoculated area died back. As long as the inoculated limb was kept under moist conditions, by enclosing it in a chimney the ends of which were plugged with wet cotton wool, the spinnengewebe mycelium spread rapidly and developed the characteristic pink colour ; but soon after the covering was removed and the infected limb was exposed to dry conditions the growth of the spinnengewebe mycelium was checked, the white or pink colour gradually faded ; small cankers developed where formerly gum had exuded. Some months after these cankers were formed, sections were taken through the callus ring round them and through the bark of the fork where the spinnengewebe mycelium had spread from the inoculum ; the sections showed the presence of inter-cellular hyphae as found in the naturally infected plant.

The inoculated plants were kept under observation for two seasons ; there was no further visible progress of the pink disease except that the inoculated limb ultimately dried up ; not only was there no sign of the disease extending from the infected limbs to the healthy limbs of the inoculated plants but there was also no spread of the disease to the other uninoculated orange and lemon potted plants kept close together in the same plant house.

Control measures

The question of the control of pink disease of orange in Balaghat district is of importance if orange cultivation is to be encouraged.

In Balaghat, citrus orchards are very near forests and therefore there seemed to be the probability of the infection spreading to the citrus trees in the wet season from diseased forest trees outside the orchards. Therefore

search was made for two seasons for pink disease infected trees in the forest near the orchards. Only two trees were found to be diseased and these too not very badly ; one was a mango (*Mangifera indica*) tree, and the other was a jack fruit (*Artocarpus integrifolia*) tree. It therefore seems that the forest trees are not a serious source of infection to the neighbouring citrus orchards, and that the infective material is present in the citrus orchard itself.

Preventive spraying and pruning of diseased parts have been found to be effective for the control of this disease not only on shrubs, like tea and coffee, but also on *Hevea* (rubber) and citrus trees. In Balaghat, pruning of dead limbs and spraying before the break of the rains have not always given satisfactory results. At first, when the trees were young, these operations could be satisfactorily carried and so the results were encouraging, but with the annual increase in the number and size of the branches there were increasing difficulties in carrying out these two operations satisfactorily. We have seen that the pink disease fungus on orange trees develops pustules and cellular aggregates inside the plant tissues, and that they remain well protected by cuticulerized and suberized cells of the bark ; this protective covering may very probably help these bodies to lie dormant during the dry season ; and the spray liquid would not reach these dormant bodies. During the wet season they are capable of becoming active and are then potential sources of infection. The infective material may be either *Necator* spores developed from the dormant pustules, parts of pustules or of cellular aggregates attached to the exfoliating bark which can be carried from tree to tree and from branch to branch by rain water, wind or insects. Spraying might protect the tree from catching the infection from these sources, but to be effective the spraying would have to be repeated very often and the forks especially would have to be kept well covered with a fungicide. It is improbable that spraying, as a prophylactic measure, would in itself be very effective in the control of this disease. As long as there are in the orchard plants with dormant infection spraying would only partially control the spread of the disease from the infected trees of the previous season. Therefore, during the dry season it would be advantageous to go over the infected trees individually and examine them for the presence of cankers. These cankers should be carefully scraped and the wounds dressed with a suitable fungicide like Bordeaux paste or preferably creosote oil. Since the fungus has been found in the tissues of the bark of a fork, the forks of infected trees, especially those formed by the main branches, should also be scraped and dressed with a fungicide.

In the wet season, limbs of the tree as soon as they are found to be diseased should be cut back much beyond the extent of the infection to ensure the complete destruction of the parasite ; the trees should be examined at short intervals for new cankers and fresh pustular growths, which should be scrapped to prevent the fungus from establishing itself on them. The wounds should be dressed with Bordeaux paste or creosote oil.

Summary

Pink disease of orange trees in the Central Provinces, caused by *Corticium salmonicolor* B. & Br., is described. It is usually in an epidemic form in Balaghat district ; it is not widely distributed throughout the province.

On orange trees the following forms of the fungus have been observed ; the spinnengewebe form, sterile pustular forms, the *Necator* form and the basidial form.

The spinnengewebe mycelium is mostly superficial ; it penetrates the bark only through wounds. Over thin-walled lenticels and over broken tissues of the bark the hyphae form loose aggregates of cells.

The sterile pustules are either white or pink or orange-red coloured. The white pustules develop either superficially on the outside of the bark or from within the bark tissues. The pink or orange-red pustules originate in the sub-epidermal tissues of the bark.

The *Necator* pustule resembles the sterile pustules till it develops spores. Spores are formed by the cells of the pseudoparenchymatous tissue of the pustule separating from one another ; the spores are one-celled, hyaline, and very variable in size and shape.

The basidial form is rare. Basidia are either formed in a row from the hymenium or are scattered ; they are sterile and plurinucleate ; sterigmata are absent.

Dormant mycelium has been observed in the callus formed round cankers and in the bark tissues of a fork.

Inoculations of orange and lemon trees have been successful in Nagpur. The infection from the successfully inoculated plants did not spread the following wet season to the healthy plants in the same plant house ; the infection did not even spread from the diseased limb to the healthy limbs of the same plant.

Climatic conditions may be the cause of the disease being in an epidemic form in Balaghat district, where there are only a few orange gardens, and the disease being sporadic and confined to only a few isolated plants in other places where orange gardens are numerous.

Control measures are suggested.

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THE SOFT-ROT OF APPLE FRUIT IN KUMAUN

BY

U. B. SINGH, M.Sc., Assoc. I.A.R.I.

Government Fruit Research Station, Chaubattia

(Received for publication on 30 April 1941)

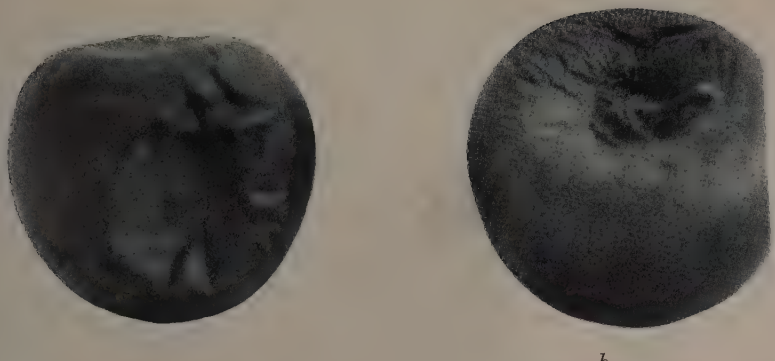
(With Plates LXIV and LXV)

A STORAGE disease of apple fruit caused by *Penicillium expansum** Lk. was first recorded in Chaubattia (United Provinces) in 1934. Kheswalla [1936] recorded it from Baluchistan. It is known in all apple-growing countries and can cause 75 per cent loss of apple fruits in storage. The earlier workers referred any blue mold attacking apples to *Penicillium glaucum* Lk. and this name is still retained by some writers even at the present time. But now it has been recognized that *P. expansum* Lk. is the most common and destructive of the various species of *Penicillium* known to attack apple fruits in storage. It is variously called soft-rot, blue-mold, bin-rot and *Penicillium* rot. There is a peculiar and characteristic musty odour, which is invariably present in diseased fruits. This odour is the first noticeable feature of this fruit decay, but the softness of the affected tissues is significant character of the disease and hence its common name is soft-rot.

Symptoms of the disease

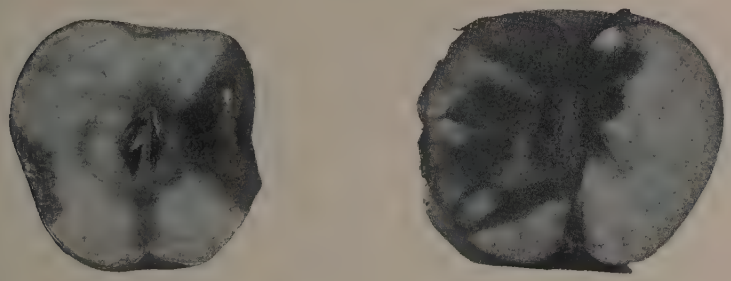
The characteristic musty odour given off from apples affected with soft-rot is a very accurate diagnostic symptom so far as determining the presence of the disease in a lot of fruit is concerned. The rotted area turns soft and watery, light or yellowish brown in colour. In lesions where a considerable portion of the apple is involved the skin becomes wrinkled, sometimes in a concentric manner. Young spots may begin anywhere on the surface of the fruit wherever there is the slightest injury in the skin (Plate LXIV, fig. 1*a*). Diseased spots sometimes start from stem-end of apple (Plate LXIV, fig. 1*b*). On cutting through the rotted area of the apple it is found to be light brown in colour and watery (Plate LXIV, figs. 2*a* & *b*). The rot is primarily one of the ripe fruits and increases with the ripeness of the fruits ; green fruits as a rule are not affected. The fungus gains entrance often through the stem-end and less frequently through the calyx-end. Under conditions of very high relative humidity a bluish green sporulating growth, which is nearly snow-white in its initial stages appears (Plate LXIV, figs. 3*a* & *b*). Hence the two characteristic diagnostic characters of *P. expansum* Lk. are (1) musty odour and (2) the formation of conidial tufts or coremia on the surface of well-developed lesions. This fungus was observed on almost all the varieties of apple grown in Kumaun.

* The culture of the fungus was sent to Mr S. F. Ashby, the former Director of the Imperial Mycological Institute, Kew, England, who identified it as *Penicillium expansum* Lk.



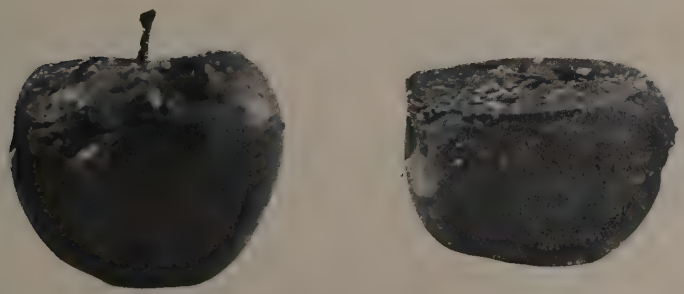
a *b*

FIG. 1. Soft rot (*a*) on Delicious apple, (*b*) at the stem end of Delicious apple



a *b*

FIG. 2. An apple (Delicious) cut open showing internal rotted area



a *b*

FIG. 3. Fructification of the fungus on the surface of the fruit

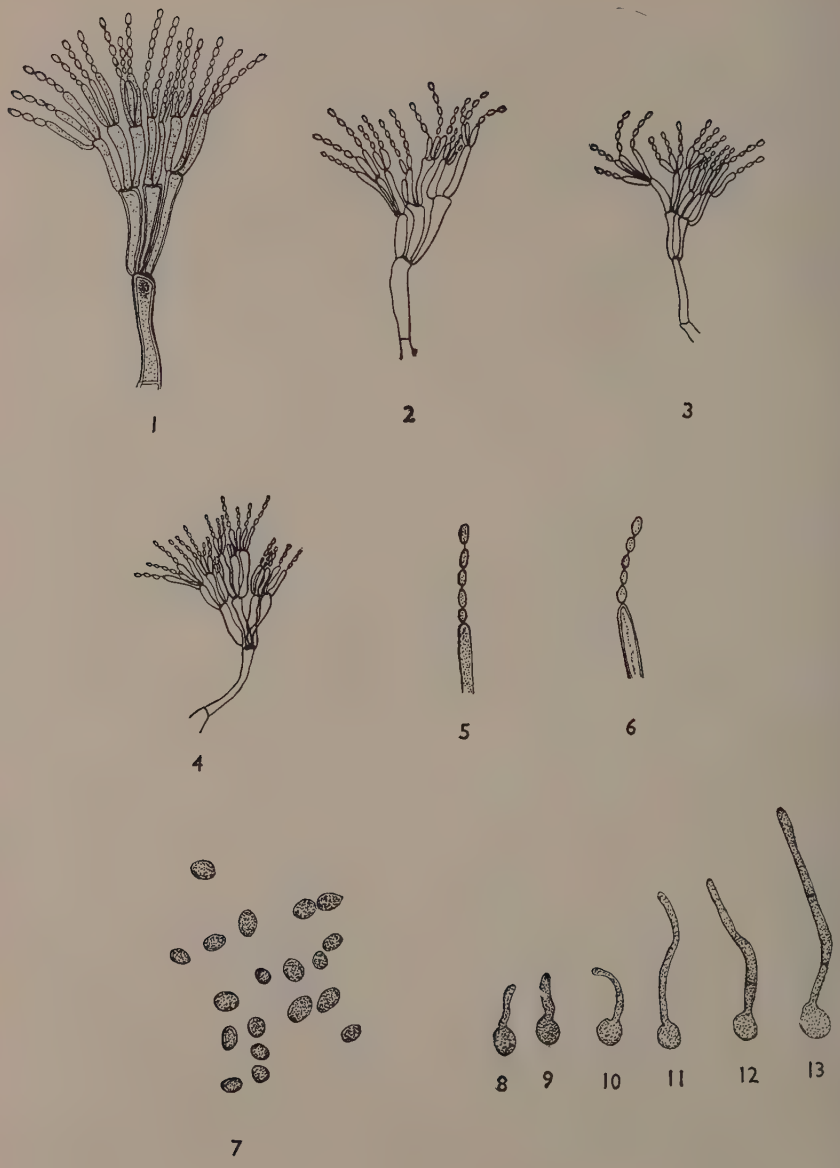


FIG. 1. Conidiophores with conidia in chains (×1,066)
FIG. 2, 3 & 4. Conidiophore with conidia in chains (×480)
FIG. 5 & 6. A single conidiophore with conidia (×1,066)
FIG. 7. Conidia (×1,066)
FIG. 8-13. Conidia germinating (after 48 hours at 20°C.) (×1,066)

Morphology

The greenish cushions or pustules which appear on the surface of the fruit are tufts of fruiting stalks of the fungus which arise from the mycelium within. A number of hyphae grow in erect fashion at the same point ; their general arrangement is like that of an inverted broom without the handle. The tips of these hyphae, or conidiophores, become branched in a digitate fashion, and at the end of each stalk is developed a chain of spores or conidia (Plate LXV, figs. 5-6). The conidiophores of the fungus are irregularly penicilliately branched (Plate LXV, figs. 1-4). Conidia are catenulate, hyaline or coloured. They measure $2 \cdot 1—4 \cdot 9 \times 2 \cdot 1—4 \cdot 4 \mu$, the average being $3 \cdot 7 \pm 0 \cdot 03 \times 3 \cdot 69 \pm 0 \cdot 01 \mu$ in diameter (Plate LXV, fig. 7). These conidia readily germinate in tap water at room temperature (Plate LXV, figs. 8-13).

Pathogenecity

The pathogenecity of the fungus was proved by means of a series of inoculation experiments by the (*a*) Granger and Horne [1924] cork-borer method and (*b*) by causing injury to the skin of apple fruit aseptically by means of a flamed scalpel and then inoculating. The fruits selected for inoculation were perfectly sound and were all of one variety (Bramley's seedling), size and maturity. These fruits were surface sterilized by dipping them in potassium permanganate solution (2/1,000) for 20 minutes then washed thrice with sterilized water and the surface wiped with absolute alcohol. For the cork-borer method ten fruits were selected, eight were inoculated with the fungus and two served as control ; the latter ones were inoculated with sterilized distilled water. Each fruit after inoculation was wiped with absolute alcohol and then wrapped with sterilized wax paper and sealed with bees wax. All these fruits were kept inside a small glass cage kept in the laboratory. In 24 days all the inoculated fruits completely rotted away with the formation of fructification on the surface in some fruits while the control ones remained unaffected. The fungus was re-isolated from inside the rotted fruits and resembled the parent culture in all respects. For the other method eighteen fruits of Bramley's seedling were at first surface-sterilized in the same way as described above. Out of these, six fruits were kept in a sterilized dish and injured at a number of places, while six fruits were kept in another sterilized glass dish but were not injured. Similarly, six fruits were injured and six were left uninjured into two separate glass dishes and these served as control. A heavy spore suspension was sprinkled by means of an atomizer in the first two sets (injured and uninjured) while sterilized distilled water was sprinkled over the control ones (injured and uninjured). On the fourth day light yellowish brown spots began to appear round about injured surfaces of apple, but there was no sign of infection in the uninjured fruits. By the eighth day all the injured fruits were completely rotted due to infection from spores. There was no infection in the control fruits. Thus it is clear that the fungus *Penicillium expansum* Lk. is a weak parasite and cannot enter through uninjured surfaces of apple fruits.

Methods of control

The onslought of this obnoxious storage disease can very well be minimized by the grower and the dealer if the following methods of control are adhered to.

Since the fungus is a wound parasite, all attempts should be made to avoid injuries.

(1) In order to avoid injuries during picking apples, the finger nails of the picker should be short or they should put on smooth gloves.

(2) Prevention of the skin injury during grading. The grader should be so adjusted that no fruit falls upon another, or rolls so as to come into violent contact with another, or with its stem.

(3) During packing the fruits should not be forced upon one another, because in doing so often the stem of one fruit injures the epidermis of another. The fruits should be wrapped with oil paper (double boiled linseed oil) and in packing the pad of the wrapping paper should be so placed as to act as a cushion and thus minimize the danger of bruising.

(4) Removal of all fruits showing the least signs of skin injury or blemish or rotting. These fruits are likely to form spores on the surface of the fruits which will act as a source of infection to other fruits.

(5) Spray treatments are useless, for no spray treatment on the trees will counteract injuries caused through subsequent careless handling.

Discussion

The soft-rot of apple fruits is the worst storage disease and can cause 75 per cent rot of apples. The fungus is omnipresent, but the fact that it cannot enter through the healthy skins of the apple suggests that the method of controlling this disease lies in the careful handling of the fruits.

The possibilities of the bruise caused at the picking by pickers with large nails are obvious and can be avoided by instructing the pickers to cut their nails or make them put on soft gloves. In packing, the fruits should be wrapped in oiled wrappers and the packing must not be too tight to cause bruises. In grading, the grader should be so adjusted that one fruit must not fall violently over the other or its stem. The removal of every fruit showing the rotting is a very effective method of controlling the disease, for, if the affected fruit is left there, it will surely, in course of time, form fructifications on the surface of the fruit and thereby cause infection to neighbouring fruits Spraying the fruit is useless.

Summary

(1) The fungus causing soft-rot disease of apples in storage in Kumaun is identified as *Penicillium expansum* Lk.

(2) The symptoms of the disease are its characteristic musty odour with rotted watery light or yellowish brown areas. Under conditions of high humidity bluish green sporulating growth appears on the surface of the rotted fruit. The fungus also starts from the stem-and calyx-ends.

(3) The morphology of the fungus is described in detail in the text. The conidiophores are irregularly penicilliately branched. Conidia are catenulate, hyaline or clear coloured.

(4) A single spore culture of the fungus was obtained. The fungus was inoculated into the healthy apple and again re-isolated and resembled the parent culture in all respects.

(5) The infection experiments showed that the fungus cannot enter through uninjured healthy skin of the apple.

(6) The fungus is a saprophytic one and is found growing on a large number of dead organic materials and produced vast number of spores. These spores float in the air and if they happen to settle down on injured surfaces of apple, they cause rotting.

(7) The effective methods of control consist in carefully handling the fruits so as to avoid injuries during picking, grading and packing the fruits.

Acknowledgements

My sincere thanks are due to Mr P. K. Dey, Plant Pathologist to Government, United Provinces, for guiding the work and the Imperial Council of Agricultural Research for financing the scheme of Hill Fruit Research Station, Chaubattia. Gratitude is expressed to Mr S. F. Ashby, the former Director of the Imperial Mycological Institute, Kew, Surrey, England, for kindly indentifying the culture.

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FATAL TEMPERATURES FOR THE PINK-BOLLWORM [*PLATYEDRA GOSSYPIELLA* (SAUND)]OF COTTON

BY

HEM SINGH PRUTHI, PH.D. (CANTAB.), F.N.I., F.R.S.B.

Imperial Entomologist

AND

TASKHIR AHMAD, PH.D. (CANTAB.)

Assistant Entomologist, Imperial Agricultural Research Institute, New Delhi

(Received for publication on 23 July 1941)

THE pink-bollworm, *Platyedra gossypiella* (Saunders), of cotton has come into great prominence during the last two decades in almost all the cotton-growing tracts of India, excepting the extremely hot and dry areas of the south-western Punjab.

Preliminary surveys carried out in the United Provinces in 1921 established beyond doubt that the pink-bollworm had become a major pest of cotton in that province. With financial aid from the Indian Central Cotton Committee, the Entomologist to the Government, United Provinces, investigated various methods of controlling the pest for several years (1925—32) and came to the conclusion that the most effective method of control was to prevent the carry-over of the hibernating larvae inside the harvested seeds by effective heat treatment [Richards, 1938]. He also reported that exposure of the infested seed to sun for a couple of hours in the afternoon (1—3 P.M.) during April and May in the United Provinces was sufficient to kill all the larvae resting within the seeds.

On the basis of this work, sun-heat treatment of cotton seed was carried out on a large scale in certain districts of the United Provinces. Although sun-treatment is a measure involving little expense beyond manual labour, its value is very limited. Its effect may be lowered by several factors such as wind, clouds, etc. which are beyond human control. Again, while the method is easily applicable to small quantities of cotton seed, it is hardly practicable in the case of large stocks usually handled under factory conditions. Moreover, during the greater part of the ginning season, the sun temperature is insufficient to kill the caterpillars, as it synchronizes with milder weather conditions. On account of these difficulties, the use of the Simon heating machines was advocated for large-scale operations in factories. The capital cost of a suitable heater and its accessories was, under pre-war conditions, about Rs. 10,000, which small factories cannot afford. Before advocating legislation that all factories in the province should provide themselves with Simon heating machines, the Director of Agriculture, United Provinces, sought the advice of the Imperial Entomologist, Imperial Agricultural Research Institute, New Delhi.

Although a fair amount of data on the lethal temperatures for this pest was available from the work done in other countries, it was thought that these results may not be exactly applicable to the larvae adapted to our climatic conditions. It was, therefore, considered desirable to investigate the behaviour of the pest to different heat treatments under Indian conditions.

The objects of this enquiry were briefly to determine :—

(*i*) The effect of exposing naked larvae to various constant high temperatures on their viability.

(*ii*) Similarly, the effect of exposing infested seeds of cotton to various temperatures on the mortality of larvae hibernating within.

(*iii*) Part played by relative humidity of air in determining the fatal effect of temperature on larvae.

(*iv*) Influence of sun's heat on the viability of larvae inside seeds.

(*v*) Effect of the above treatments on the viability of cotton seed.

(*vi*) Maximum exposures to various high temperatures tolerated by the seed.

(*vii*) Effect of variety of cotton seed on the larval mortality under various conditions of heat treatment.

We wish to express our appreciation of the help received from Mr Ghulam Ullah, Assistant to the Imperial Entomologist, in carrying out experimental work with keen interest and great care.

Review of previous work

In view of the possibilities of heat treatment being a useful control measure against pink-bollworm, considerable work has been done on the fatal temperatures for this insect in several cotton-growing countries of the world. Gough and Storey [1913], working in Egypt, dipped infested cotton seeds in water maintained at different temperatures. They found that dipping the seeds for five minutes in water at 50°C. killed 97 per cent of the larvae, while dipping for two minutes at 55°C. gave complete larval mortality. The germination of seeds was not affected. The viability of seeds was only affected (about 20 per cent) when they were dipped for five minutes in water at 75°C. Similarly, seeds exposed to hot air in a chamber, the tempeature of which was roughly controlled, gave the following results :—

Air temperature in the chamber (°C.)	Exposure (minutes)	Larval mortality (per cent)
69—80	5	Partial
About 80	4	100
63—72	10	75
75—94	10	100

Later on, Storey [1915], as a result of his large-scale experiments performed in better insulated machines in which the seed could be exposed in thin layers over several circulating canvas belts, got the following figures :—

Air temperature (°C.)	Exposure (minutes)	Larval mortality (per cent)	Remarks
About 60	10·5	100	Germination not affected
60—70	6·5	100	Do.
70—80	3·5	100	Do.
85—90	3·5	..	Evidence of germination being affected

He further noticed that temperature remaining the same, if the intake of seeds was increased so that seeds were exposed in thicker layers, the rate of mortality was lowered.

Similar experiments were carried out by Gough [1916] in a hot machine, the temperature inside which was controlled by the opening or closing of a damper placed between the hot air generator and the chamber. His results are summarized below :—

Chamber temperature (°C.)	Temperature (°C.) of seed at the exit	Exposure (minutes)	Larval mortality (per cent)
75	47	9	96
80	50	9	100
80	48	7	97
85	50·5	7	100
80	..	5	38
90	47·75	5	100

It will be observed that there was great difference between the temperature inside the machine and that at its exit. As a result of these experiments, Gough's chief conclusion was that to ensure complete larval mortality, the temperature of the chamber and exposure of seeds should be so regulated that the temperature of the seed at the exit is about 50°C. He admitted that at some points in the chamber close to the hot pipes the temperature went as high as 170°C. (which is extremely lethal) but he argued that as the

seeds came into contact with such places only for a moment or so, no harm was done to their viability.

The experience of Storey [1916] on the treatment of cotton seed in the Simon's hot-air machine made it clear to him that obtaining a certain requisite seed temperature at the exit was not enough. He found that all minute details in exposing, handling and final disposal of the seeds had to be taken into account to determine the final effect of the treatment. For instance, in practice, the temperature of the machine was maintained at 55°—56°C. and the seeds passed through it in about five to seven minutes. After coming out, if the hot seeds were put into sacks and kept aside for some time, complete mortality among the larvae was observed. On the other hand, if after the hot treatment, the seeds were not put into sacks but were allowed to cool in the open, several larvae survived. This showed that sacking or otherwise handling the seed subsequent to heating inside the machine had important influence on the result of heat treatment. Obviously, sacking resulted in the maintenance of, if not further rise in, temperature which ensured complete mortality of the larvae.

The methods of heating cotton seed evolved in Egypt were however not found to be equally successful in Texas (U. S. A.), owing probably to the different nature of the seed, the behaviour of the insect, etc. [McDonald and Scholl, 1922]. As a result of several experiments, these authors concluded that the seed must be exposed to a higher temperature inside the machine than that which was to be obtained at the exit. Under strictly controlled conditions although an exposure of three minutes to 58°C. gave complete mortality, in practice seeds had to be uniformly heated at 63°C. for 3½ minutes to free them of all living bollworms. Further, they found that it was possible to heat the seeds up to 74°C. for a short while without injuring their germination. However, seeds could survive much higher temperatures provided the exposure was less. For instance, a germination of 93 per cent was observed after an exposure of one minute to 160°C.

Bredo [1934], reviewing the work carried out in Egypt, Belgian Congo, etc., concluded that a machine without an automatic feeder was inefficient, as heat penetrated imperfectly if the seeds were admitted in a thick layer. He further confirmed Gough's observations that when the temperature at the exit of the machine was 50°C., that of the interior might be above 100°C. Again, the exit thermometer might register 65°C. if the seed was dry and 60°C. if it was moist. Commenting upon the Egyptian experiments, he stated that exposure of five minutes and exit temperature of 55°C. proved effective only if the seed was subsequently kept in sacks for a period of two hours. If the seed was not put into sacks immediately, its temperature at the exit must be at least 65°C. for the same length of exposure.

With regard to the use of Simon heater in the United Provinces, Richards [1938] concluded that it was essential to so regulate the steam input that the seed at the exit reached a maximum temperature of 60°C. It was further found that dry seed in a dry atmosphere retained its normal viability up to 82°C. In the presence of moisture, however, some loss of seed viability was observed even at 63°C. A temperature of 62°C. was, therefore, considered as the maximum to which seed should be actually heated.

From this brief review of previous work it will be obvious that exact temperature requirements to get complete larval mortality depend on a large number of factors. For instance, the thickness of layer in which seeds are passed through the heating machine, the extent of stirring, the insulation of the machine, the duration of exposure, the temperature of the atmosphere from which the seeds are brought and to which they are taken after treatment all affect the temperature to which seeds get actually heated. Moisture plays an important part. Apart from its effect on larvae, which was hitherto not clear, Bredo [1934] showed that machine temperature, and the process and duration of exposure remaining the same, dry seed registered 5°C. higher temperature at the exit than the moist seed, showing thereby that dry seed got heated quicker than the moist seed. In the same way the combined effect of temperature and humidity on the viability of seed has to be carefully kept in view. According to Richards [1938] moist seed is more susceptible to high temperature than dry seed. It was probably for such reasons that the methods evolved in Egypt, when tried in Texas were not found to be equally successful. It is, therefore, clear that the results obtained on fatal temperatures for the pink-bollworm vary widely under different environmental conditions of the country and the machine in which treatment is carried out, and results obtained in one country must be tested in the other country before they are adopted for general use. In fact the detailed procedure of large-scale treatment must be standardized for each country.

Material and method

The cotton crop of 1939-40 on the estate of the Imperial Agricultural Research Institute was badly infested with pink-bollworm and afforded abundant material for our investigation. About two maunds of cotton seed of indigenous Mollisoni variety and three maunds of American variety (289 F) were examined soon after the harvest and double seeds (containing the larvae) separated. Almost all the larvae were full grown and appeared to be in a state of hibernation.

At first, preliminary experiments were carried out by placing the double seeds in a copper or glass tube about one inch in diameter, dipped in hot water maintained at a constant temperature, with a fluctuation of not more than $\pm 0 \cdot 25$°C. It was noticed that the air space between the seeds acted as a fairly good insulator in the transmission of heat from the wall of the tube to the centre of the seed mass. A thermometer fitted in a cork was placed in the centre of the seed mass in the tube. The rate of rise in temperature was noted every minute and the records are given below :—

Table I

Rate of rise in temperature of cotton seed placed in a tube immersed in hot water

Temperature of water (°C.)	Container	Temperature (°C.) of seed recorded every minute								
		1	2	3	4	5	6	7	8	9
68	Copper tube	42	52	59	62	64	65·5	66·5	67·5	67·5
	Thin glass tube.	42	51	57	59	64	65	67	68	68

It will be noticed that surface temperature of seeds in the centre of the tube reached within a degree of the water temperature in seven to eight minutes. Thus the temperature of the larvae within the seeds would take a still longer time to reach that of the water bath. There was, however, little difference between a copper and a glass tube at the end of this period, although in the beginning the copper tube got hotter much more quickly than the glass tube.

In order to reduce this error to the minimum it was decided to expose the seeds to high temperature by spreading them in a single layer. This was done inside a thermostatically controlled hot oven. Thus the seeds, immediately after the exposure, came in touch with an atmosphere already at the desired temperature. This reduced the interval which heat took to penetrate from the water bath to the centre of the tube in the previous experiments. A sensitive thermometer placed with its bulb in contact with the seed layer showed that the seed acquired the temperature of the oven, which was 70°C. in a particular experiment, in three minutes. When the seed was introduced into the oven, the temperature of the latter fell down by one degree. As the extent of fall and the time for which it lasted are extremely small, it may be claimed that results obtained with these experiments are fairly reliable. The effect of this fall will be still more insignificant in experiments involving exposures longer than five minutes, because the temperature adjusted itself within the first three minutes.

These experiments also suggest that in practice not only must the seed be exposed to hot air in a very thin layer but the air must be kept in motion. In a calm atmosphere the seed absorbs heat from the surrounding layer, the temperature of which consequently drops. Now as the air is a bad conductor of heat the drop in temperature of the layers of air immediately surrounding the seed is not made up at once and in practice the seed remains exposed for some time to a temperature lower than that of the main atmosphere inside the chamber. But if the air is in motion every moment fresh air at the required temperature strikes the seed surface the old cooler air being constantly taken away and warmed up.

Fatal temperatures for naked larvae

The actual temperature to which the larvae themselves are exposed and as a consequence of which they die as distinct from the temperature to which the seed is exposed is of fundamental importance and forms the basis of heat treatment; yet little work has been done to test the naked larvae (taken out of the seed) with regard to their heat resistance. In almost all previous investigations workers have exposed larvae enclosed in seeds to different temperatures. Although this is the manner in which larvae are to be subjected to heat treatment in practice, it must be realized that temperature of the hot air to which seeds are exposed remaining the same, the temperature attained by the larvae within the seeds, which is of real importance, depends on several other factors, such as the temperature of the seeds before exposing to heat, the thickness and hairyness of the seed, its moisture content, the temperature to which seed is brought back after treatment, etc. For instance, seed at an original temperature of 10°C. may require double the exposure to

a given high temperature to effectively kill all the larvae inside them as compared with another lot at an initial temperature of say 40°C. It is, therefore, essential to know in the first instance the temperatures and their durations which are fatal to naked larvae themselves.

In the case of moderately high temperatures of 45°C. and 50°C., larvae were exposed under controlled humidities on a glass plate inside desiccators as explained in the next section and the results are set forth in part A of Table II. At 50°C., and higher temperatures, where the exposures were short, the humidity was not controlled and larvae were exposed on a copper plate in the following manner :—

A known number of naked larvae were placed in a double-walled copper container. The space between the two walls was filled with water at the temperature to be tested and the container placed in hot oven at the same temperature. The larvae were thus in contact with hot copper wall which readily transmitted its heat to them and at the same time recouped its loss by taking heat from water inside the container. The air surrounding the larvae was also at the required high temperature of the oven. The results thus obtained are summarized in part B of Table II.

It will be observed that an exposure of naked larvae for about 24 hours to 45°C., 1—2½ hours to 50°C., 7—10 minutes to 55°C., five minutes to 60°C., two to three minutes to 65°C. or one minute to 70°C. proved completely fatal. Thus, in practice, the seed should be so treated as to ensure that the larvae within the seed get the necessary exposure to any particular temperature mentioned above. The longer exposures are, however, impracticable from commercial point of view as time factor is of great importance in dealing with large quantities of material. An exposure of larvae for two to three minutes to a temperature of 65°C. or for one minute to 70°C. may be considered ideal from practical point of view.

The only previous work which may be said to approximate to our experiments with naked larvae described above is that of Gough and Storey [1913]. These authors dipped seeds containing the larvae directly into water maintained at constant temperatures and found that dipping for two minutes in 55°C. or for five minutes in 50°C. was almost completely fatal. In these experiments larvae may be said to have attained the temperature of the hot water almost immediately after dipping, as the water penetrates into the infested and consequently damaged seeds at once. But it will be readily appreciated that these experiments are very different from ours, because of the actual immersing of the larvae in hot water, which apart from its effect due to temperature, influences the larvae due to partial or complete drowning. In all other experiments reported in literature, double seeds have been exposed to hot air and, as already shown, larvae inside the seed take 8—15 minutes to acquire the temperature of the hot air. It is obvious that when the exposures are less than 8—15 minutes, the larvae inside the seeds never attain the temperature of the surrounding air. That is why the actual temperature of the seed (at the exit of heating machines) is far lower than that prevailing inside the machine chamber.

Table II

Mortality in pink-bollworm larvæ exposed naked for different durations to different constant temperatures and saturation deficiencies

	Temperature (°C.)	Saturation deficiency (mm.)	Exposure	No. of larvæ Exposed	No. of larvæ Died	Percentage mortality	Remarks
A	45	3	12 hours .	30	17	56·7	Larvæ exposed on a glass plate
			24 ,, .	20	20	100	Do.
		14	12 ,, .	30	22	73·3	Do.
			24 ,, .	20	20	100	Do.
		32	12 ,, .	30	7	23·3	Do.
			24 ,, .	20	17	85·0	Do.
	50	3	2½ ,, .	30	30	100	Do.
			4 ,, .	30	30	100	Do.
		14	2½ ,, .	30	30	100	Do.
			4 ,, .	30	30	100	Do.
		32	2½ ,, .	30	22	73·3	Do.
			4 ,, .	30	30	100	Do.
B		..	10 minutes	12	0	0	Larvæ exposed on a copper plate
			30 ,,	30	16	53·3	Do.
			45 ,,	45	45	100	Do.
	55	..	3 ,,	12	2	16·7	Do.
		..	3 ,,	12	10	83·3	Do.
		..	5 ,,	12	11	91·7	Do.
			7 ,,	50	50	100	Do.
			10 ,,	20	17	85·0	Water bath*
			20 ,,	20	20	100	Do.*
	60	..	1 minute	74	57	77·0	Larvæ exposed on a copper plate
			3 minutes	37	32	86·5	Do.
			5 ,,	20	20	100	Water bath*
	65	..	1 minute	87	85	97·7	Larvæ exposed on a copper plate
			3 minutes	25	25	100·0	Do.
	70	..	1 minute	50	50	100·0	Do.

* With the exception of these experiments all others were conducted in the incubator (See 'Material and method')

INFLUENCE OF ATMOSPHERIC MOISTURE ON LARVÆ EXPOSED TO HIGH TEMPERATURES

The part played by the atmospheric moisture in causing larval mortality of *P. gossypiella* at high temperatures has not been investigated so far, although some observations on the effect of moisture on the viability of cotton seed [Richards, 1938] and on the rate at which heat is absorbed by the seed [Bredo, 1934] are available. In order to investigate this aspect of the problem, saturation deficiencies (S.D.) of approximately 3 mm., 14 mm. and 32 mm. were maintained in desiccators with various saturated salt solutions, at constant temperatures of 45°C. and 50°C., as detailed in Table III.

TABLE III

Relative humidities obtained at various constant temperatures by the use of saturated salt solutions

Temperature (°C.)	Saturation deficiency	Salt solution used	Relative humidity obtained (per cent)
45	3 mm.	K_2SO_4	97
	14 mm.	KCl	81
	32 mm.	$NaNO_3$	56
50	3 mm.	K_2SO_4	97
	14 mm.	KNO_3	84
	32 mm.	$NaNO_3$	62

Naked larvae were exposed as in previous experiments in dishes inside the desiccators. It may be added that the desiccators were placed in the incubator several hours before the larvae were introduced to ensure that the temperature inside the desiccator was the same as that of the incubator and that the required relative humidity had also come into equilibrium with the temperature. The results of these experiments are summarized in Table II.

It is interesting to note that while an exposure of 24 hours to 45°C. is completely fatal under S. D. of 3 and 14 mm., it is not so under 32 mm. Likewise, an exposure of $2\frac{1}{2}$ hours to 50°C. is completely fatal under S. D. of 3 and 14 mm., but gives a partial mortality (73·3 per cent) if the S. D. is 32 mm. Thus, under comparatively dry conditions, the larvae resist high temperatures better; in other words, exposure remaining the same, higher temperatures are required to obtain complete mortality under dry conditions.

The practical importance of these findings is that other conditions such as temperature and exposure remaining the same, the heat treatment will be more effective during moist weather, or if the hot air of the oven is artificially

charged with moisture. It must be stated here that according to Richards [1938] cotton seeds although resistant to dry heat lose their viability quicker under moist conditions. Therefore, in heat treatment both temperature and humidity conditions have to be suitably adjusted.

It would at first sight seem peculiar that a moisture-loving insect like *Platyedra gossypiella* should resist high temperatures better under dry conditions. Haroon Khan [1938], studying the ecology of pink-bollworm in the Punjab, showed that the pest is always serious in regions having mild and humid climate and negligible in hot and dry areas. Likewise, Squire [1937 ; 1940], investigating the causes underlying the diapause in this insect, concluded that ' short-cycle ' or quick-developing larvae are found in nature as long as the environment and food are moist, but they begin to enter ' long-cycle ' phase or hibernation when the conditions become unfavourable, *viz.* the environment and the food become dry. Thus according to him, lack of moisture is the chief factor producing diapause in this species. Conversely, the diapause is broken and larvae become active when they are placed in a moist atmosphere and are supplied with moist succulent food. Heavy and regular rainfalls during early period of the cotton crop are a feature of those countries where pink-bollworm is a serious pest, as these conditions are necessary to make the hibernating larvae active which in due course attack the new crop.

A close examination of these observations will indicate that resistance to dry heat does not go counter to the moisture-loving habit of the bollworm. Whatever the temperature, under dry conditions, aestivating larvae continue to remain inactive and it is a well-known fact that a quiescent stage of an insect can resist high temperatures better than its active stage. On the other hand, under moist conditions the diapause of the larvae tends to be broken, they become active and consequently succumb quicker to the influence of a given high temperature.

Fatal temperatures for larvae inside infested seeds

The results obtained from the exposure of naked larvae to heat, although of fundamental importance, are of only indirect value from practical viewpoint, as it is invariably the seeds which are exposed to heat for killing the hibernating larvae inside. All the previous workers, therefore, have tested the influence of high temperatures on larvae enclosed within the cotton seeds. In the following pages the data obtained by us on the influence of constant high temperatures, and of sun's heat on larvae infesting different varieties of cotton seeds are discussed.

Effect of constant high temperatures on larvae enclosed in double seeds

One to two hundred double seeds, containing in aggregate 50-90 living larvae, were put in a single layer inside incubators, running at constant temperatures (45°—70°C.). Before transferring to incubators, the seeds were kept at a room temperature of 35°—40°C., and were again put at this temperature after exposure to the constant temperatures referred to above. The seeds were then opened and it was quite easy to distinguish the larvae which had died a minute or so ago on account of heat treatment from those which

were dead long ago due to other factors, before the seed was put in the incubators. The data regarding the effect of this treatment on larvae are summarized in Table IV. It was observed that the results obtained with water bath did not differ very much from those got with the incubator and are therefore also presented with necessary remarks in the same table.

These experiments indicate that for heat treatment, if seeds are brought from and taken to a room temperature of about 35°—40°C., an exposure of two hours to 50°C., 20 minutes to 55°C., 10 minutes to 60°C., 5 minuts to 65°C. or less than 3 minutes to 70°C. causes a partial larval mortality, while an exposure of a little over three hours to 50°C., 40 minutes to 55°C., 15 minutes to 60°C., 7—10 minutes to 65°C., or 3—5 minutes to 70°C. is completely fatal to the larvae.

In practice it will be better to adopt even longer exposure as the range of temperature at which the viability of seeds is affected is fairly higher as will be shown hereafter.

Effect of sun's heat on larvae inside double seeds

The sun's heat is the most important source of energy readily available without any cost. Its power to kill insects, particularly the pink-bollworm larvae inside seeds, is well known and it is a common practice with zemindars to expose their cotton seeds and other grains to sun's rays for disinfection. Richards [1938] stated that the maximum seed temperature recorded on a *pucca* floor during summer months in the United Provinces was 71°C. and a few minutes exposure to this temperature was sufficient to kill the larvae. On the other hand, on a *kucha* floor the temperature of seed seldom rose above 60°C. and for effective treatment it was necessary to keep the seeds on such floors for about ten minutes. Similarly, at temperatures between 52° and 56°C., 20 minutes exposure and at 50°C. an hour's exposure were necessary. Excepting these observations no detailed record of temperature variation from time to time of the soil and of the air and their effect on seeds are available. The intensity of the sun's heat, being often variable from minute to minute or day to day, depends on so many uncontrollable factors that it is difficult to appreciate its effect without an accurate and detailed record of all the conditions prevailing at the time.

Some of the important conditions which affect the intensity of sun's heat reaching an exposed material are :—

(*i*) The foremost factor affecting the intensity of sun's heat reaching the earth is its position and distance from the earth, which varies rapidly at different times of the day. This means that the exact geographical position of a locality and the time of the day for heat treatment are very important.

(*ii*) The amount of heat reaching the earth also depends on the condition of the medium through which it travels. Presence of dust and clouds in the way may almost completely cut away the supply of heat.

(*iii*) The velocity of prevailing wind and its humidity have also important influence.

(*iv*) Nature of floor on which an article is exposed plays an indirect but an important role in determining the rate of heating. Seeds spread in the sun on cloth or on *kucha* floor are exposed to much less heat from below than those spread on brick, cement, stone or metal floor.

TABLE IV

Effect of constant high temperatures on larvæ inside seeds of American cotton (289 F.)

Temperature (°C.)	Exposure	No. of larvae		Mortality (per cent)	Remarks*
		Exposed	Died		
50	2 hours	33	27	81	
	3 hours 10 minutes	30	30	100	
	4 hours	52	52	100	Th.
55	20 minutes	54	36	66·7	Inc.
	40 ,,	32	32	100	,,
	20 ,,	45	32	71·1	Th.
	40 ,,	48	48	100	,,
	40 ,,	43	43	100	,,
60	10 ,,	38	29	76·3	Inc.
	15 ,,	50	50	100	,,
	5 ,,	22	15	68·2	Th.
	5 ,,	48	10	20·8	,,
	7 ,,	62	48	77·4	,,
	10 ,,	13	13	100	,,
	10 ,,	53	53	100	,,
61·6	10 ,,	46	43	93·5	Inc.
	14 ,,	42	42	100	
65	5 ,,	34	13	38·2	,,
	10 ,,	38	38	100	,,
	5 ,,	112	97	86·6	Th.
	7 ,,	50	47	94	,,
	7 ,,	50	41	82	,,
	10 ,,	51	51	100	,,
69·2	5 ,,	49	14	29·4	Inc.
	7 ,,	35	35	100	,,
70	5 ,,	17	17	100	,,
	3 ,,	19	19	100	,,
	3 ,,	45	23	51·1	Th.
	3 ,,	64	34	53·1	,,
	5 ,,	58	58	100	,,
	5 ,,	50	37	74	,,
	5 ,,	45	33	73·3	,,

* Th. = observations taken in the water bath; Inc. = observations taken in the incubator

The present experiments were conducted at the Imperial Agricultural Research Institute, New Delhi, during June 1940 and March and May 1941 under as normal conditions as possible. Seeds were spread in a single layer on a *kucha* floor at different times of the day. It was observed that while the temperatures of the atmosphere at 10 A.M. and 5 P.M. were almost similar, those of the ground below were very different (Table V) and, therefore, different results were obtained. It was also evident from these experiments that when the shade temperature was between 38° and 40°C. and that of the soil in the sun between 50° and 55°C., under normal summer sunshine in June (Experiment No. 10), an exposure of seeds to sun on a *kucha* floor for 34 minutes gave complete larval mortality. Likewise, during hotter part of a similar day when shade temperature was 42°—43°C. and soil temperature in the sun between 60° and 65°C. (Experiment No. 14), an exposure of seeds for only about ten minutes was sufficient to kill all the larvae in double seeds. On the other hand during March when the temperature in shade was 26°—31°C. and soil temperature in the sun was between 34°—48°C. (Experiment No. 1), an exposure for even three hours was ineffiective to kill the larvae. A similar exposure on a hotter day in the same month (Experiment No. 3) was completely fatal. In another experiment (No. 7) during May, a cool day preceded by a small shower of rain, was selected and double seeds were exposed to the sun as usual. During the period of exposure there were wide fluctuations of temperature on account of the cool breeze and clouds appearing at times. An exposure of larvae inside double seeds for 9 hours on that day produced partial mortality (90 per cent). This experiment points out the wide fluctuations which sun's heat sometimes undergoes. A full day's exposure to sun in May (usually the hottest month) subsequent to rain and cool breeze may be useless, while only 10 minutes' exposure on a clear still day in June (Experiment No. 14) may give complete mortality. Thus it is not enough to lay down merely that seeds should be exposed for a certain period to sun in a certain month, without warning that the factors referred to above must be kept in view while selecting a day.

Sun treatment is, therefore, not a very dependable method of disinfection, particulary in the hands of illiterate and ignorant zemindars. Even in the hands of literate people who can take all the necessary precautions, such treatment will have to be followed by actual tests of larval mortalities, as this alone is the real criterion of effective treatment.

Influence of nature of seed on the mortality of larvae under heat treatment

The nature of seed varies in different varieties of cotton. In some varieties the seed is small and compact, in others it is larger but more soft. Again, some are fuzzy while others are bare. Accordingly, when exposed to heat treatment, they offer different degrees of protection to the larvae lying within. Furthermore, as already pointed out, the quantity of moisture in the seed also greatly affects the rate at which it gets heated.

Two common varieties of cotton seed, *viz.* American (289 F) and *desi* (Mollisoni) were used for comparison and the results are summarized in Tables IV and VI. The seeds of American variety were large and hairy, those of

TABLE V

Larval mortality inside double seeds of cotton exposed to sun during March to June at Delhi

Experiment No.	Period of Experiment	Time of the day	Exposure	Temperature range °C.				Mortality per cent		Remarks
				Shade	Soil	Sun		*Desi*	American	
						Black bulb	White bulb			
1	March	9 A.M.	3 hours	26·0—31·0	34·0—48·0	38·0—60·0	30·0—41·0	0	0	Light clouds appearing every now and then
2	,,	,,	1 hour 45 minutes	30·0—34·5	38·0—53·3	47·7—65·5	35·0—46·2	69	72·2	
3	,,	,,	3 hours	30·0—37·2	38·0—59·0	47·7—67·2	35·0—49·0	100	100	
4	,,	2-20 P.M.	15 minutes	39·0—39·0	54·5—56·8	48·0—67·5	40·3—50·0	95	97	
5	,,	,,	30 ,,	39·0—39·5	54·5—56·8	48·0—68·0	40·3—49·8	100	100	
6	May	8-22 A.M.	5 hours	27·0—31·0	31·0—49·0	42·2—61·1	32·2—43·8	69	61	A cool day preceded by light shower of rain
7	,,	,,	9 ,,	27·0—33·5	30·0—54·5	40·0—67·2	32·2—46·1	90	93	
8	June	9 A.M.	35 minutes	35·0—35·7	43·6—47·0	49·0—61·0	39·5—45·5	0	...	
9	,,	,,	60 ,,	35·0—36·2	43·6—50·5	49·0—63·0	39·5—47·0	47	...	
10	,,	10 A.M.	34 ,,	38·7—40·0	49·5—55·3	62·0—67·0	47·0—51·0	100	...	
11	,,	5 P.M.	10 ,,	41·7—41·4	54·7—54·8	51·0—61·0	41·0—47·5	18	...	
12	,,	,,	20 ,,	41·7—41·2	54·7—54·8	51·0—63·0	41·0—49·0	53·7	...	
13	,,	1 P.M.	5 ,,	42·2—41·7	60·0—64·3	57·0—68·0	44·5—52·0	98	...	
14	,,	,,	10 ,,	42·2—42·7	60·0—65·7	57·0—73·5	44·7—54·0	100	...	

the *desi* variety were small and bare. But the texture of *desi* seeds was much more compact than that of the American variety.

From a comparison of Tables IV and VI it will be noticed that although there is not very marked difference in the mortality of larvae inside the two varieties of cotton seeds, there is throughout a somewhat higher mortality among larvae inside the American variety as compared with those inside the *desi* variety. Thus, for instance, while an exposure of two hours to 50°C. caused 81 per cent larval mortality in American seed, an exposure of two and a half hours to the same temperature caused 72·7 per cent mortality in the case of *desi* seed. Likewise, an exposure of 20 minutes to 55°C. caused 66·7 per cent mortality in the American, against 21·1 per cent in the *desi* variety and so on. However, as these differences are not very great, it will be quite effective to recommend for general adoption somewhat longer exposures or slightly higher temperatures to cover the varietal differences indicated above. This should be quite feasible since there is fair margin of safety between heat exposures fatal to larvae and those injurious to the viability of the seed.

Effect of high temperature on the viability of cotton seed

In order to determine the effect of different degrees of heat on the viability of cotton seed, 100 apparently sound seeds of two varieties were selected and exposed for definite periods to a number of constant temperatures. They were then sown in moist sand and kept for germination at room temperature of 35°—40°C. Almost all the viable seeds germinated within a week and their percentage was noted. The results are summarized in Table VII. In the case of the control experiments comprising four tests with Mollisoni variety and two tests with American variety, it will be noticed that unfortunately the original viability of the seeds was not very high, only about 50 per cent of *desi* and 24 per cent of American seeds being viable. This range of percentage viability is practically maintained after almost all the heat exposures tried. Thus, briefly speaking, the viability of seeds is not materially lowered after an exposure of 30 minutes to 65°C., or 20 minutes to 75°C., or 10 minutes to 80°C. This indicates that there is a fair margin of safety between the heat treatments necessary to kill the bollworm and those at which the viability of the seeds is adversely affected.

Conclusions and summary

The method of controlling the pink-bollworm by preventing the 'carry over' of the hibernating larvae found inside the harvested seed by suitable heat treatment has been followed in some countries and recommended for adoption in certain parts of India. Yet little accurate data on lethal temperatures and humidities for the bollworms themselves (outside the cotton seed) and for the seed are available under Indian conditions. At the request of the Department of Agriculture, United Provinces, this investigation was taken up in 1940 and the results of the work carried out so far are presented in this paper.

TABLE VI

Effect of constant high temperatures on larvae enclosed in double seeds of desi *cotton (Mollisoni)*

Temperature (°C.)	Exposure	No. of larvae		Mortality (per cent)	Remarks*
		Exposed	Died		
45	12 hours . . .	48	0	0	Inc.
	24 ,, . . .	53	47	88·7	,,
	36 ,, . . .	..	..	..	
50	2½ ,, . . .	66	48	72·7	Inc.
	4 ,, . .	58	58	100	,,
55	20 minutes . . .	38	8	21·1	,,
	40 ,, . . .	5	5	100	,,
	40 ,, . . .	50	50	100	,,
60	10 ,, . . .	35	5	14·3	,,
	15 ,, . . .	15	15	100	,,
64·4	5 ,, . . .	40	14	35	,,
	10 ,, . . .	51	51	100	,,
65	10 ,, . . .	1	1	..	
	10 ,, . . .	13	13	81·1	Th.
	10 ,, . . .	56	47		,,
69·2	5 ,, . . .	50	12	24	Inc.
	7 ,, . . .	12	12	100	,,
70	3 ,, . . .	27	16	59·2	Th.
	5 ,, . . .	28	14	50	,,

* Inc. = observations taken in the incubator; Th. = observations taken in the water bath

TABLE VII

Effect of high temperatures on the germination of cotton seed
(100 seeds sown in each case)

Temperature (°C.)	Exposure	Percentage germination	
		Desi	American
45	12 hours	52	..
45	24 ,,	53	..
50	4 ,,	54	..
50	6 ,,	43	..
55	1½ ,,	58	..
60	30 minutes	65	..
65	10 ,,	43	34
65	15 ,,	38	28
65	30 ,,	52	26
70	5 ,,	49	15
70	10 ,,	37	30
70	20 ,,	42	26
75	5 ,,	42	21
75	10 ,,	40	16
75	20 ,,	38	13
80	3 ,,	57	23
80	5 ,,	59	24
80	10 ,,	47	27
Control	....	54	..
,,	....	53	..
,,	....	48	20
,,	....	45	28

Hitherto almost all the workers have subjected bollworms enclosed in cotton seeds to various temperatures. It is obvious that the value of such results is limited as the effect of these exposures would vary widely with the temperature and moisture content of the environment in which seeds remain before and after treatment, the texture and fuzziness of the seed, the thickness of layer in which seeds are passed through the heating machine, etc. Thus the heat-tolerance of naked larvae taken out of the seeds which is of fundamental importance in the study of heat treatment, as a control measure, has been investigated for the first time.

These experiments have shown that naked larvae undergo complete mortality when exposed for 24 hours to 45°C., 1—2½ hours to 50°C., 7—10 minutes to 55°C., five minutes to 65°C. or one minute to 70°C. If instead of naked larvae the cotton seeds containing larvae are treated and are brought from and taken to a room temperature of 35°—40°C., in a thin layer, an exposure of a little over three hours to 50°C., 40 minutes to 55°C., 15 minutes to 60°C., 7—10 minutes to 65°C., or three to five minutes to 70°C. is completely fatal to larvae within the seeds. It may thus be concluded that from practical point of view, where time factor is of considerable importance in dealing with large quantities of material, the exposure of seeds to heat should be so regulated in reference to the initial and final seed temperature and the nature of seed, etc. that the larvae themselves inside the seeds are at a temperature of 65°—70°C. for one to two minutes.

The part played by atmospheric moisture in determining larval mortality at different high temperatures was hitherto little explored. We have now determined that under relatively dry conditions the larvae resist high temperatures better and, therefore, longer exposures would be required to ensure complete mortality. For instance, while an exposure of seed for 24 hours to 45°C. is completely fatal to larvae if the saturation deficiency of air is 3—14 mm., it is not fatal if the saturation deficiency is 32 mm.

Experiments on the protection afforded by the variety of cotton seed to the larvae inside them during heat treatments were conducted only on two varieties, *viz.* a *desi* variety (Mollisoni) and an American variety (289 F). It has been found that although the difference is not great, the heat treatment remaining the same, there was always higher mortality among larvae inside American seed as compared to those inside *desi* seed.

The viability of cotton seed is not affected materially up to an exposure of about 30 minutes to 65°C. or 20 minutes to 75°C. or 10 minutes to 80°C. This shows that there is a fair margin of safety between heat exposures fatal to larvae and those injurious to the viability of the seeds. It must be pointed out that in practice the seeds after coming out of the hot machine retain heat for some time, particularly if they are put into sacks immediately after treatment and this period must be kept in view while prescribing temperature and exposure.

The technique of heat treatment is discussed. It is experimentally shown that heat takes a considerable time to penetrate through a layer of cotton seed. For instance, when seeds were transferred from a room temperature of about 36°C. to a chamber at 68°C., the seeds only less than half an inch below the surface took seven to eight minutes to reach within a degree of the chamber temperature. These experiments emphasize that during

heat treatment, the seeds should be exposed in a very thin layer, the entire quantity should be kept well stirred constantly and the chamber-air above the seeds must also be kept in motion.

The effect of sun's heat as a method of killing larvae inside seeds is discussed. The intensity of sun's heat is so markedly affected by factors beyond human control that a warning is given here against laying down any dogmatic principles about the disinfection of seeds by exposing to sun. For instance the heat reaching an exposed material from sun depends not only on its diurnal position and distance from earth but also on the condition of the medium through which heat travels (*e.g.* the presence of dust, clouds, etc.), the velocity and nature of prevailing winds, the nature of floor on which the seeds are spread, etc. Results of some experiments accompanied with records of air temperature in shade, and in the sun (black and white bulb temperatures), soil temperature, etc. are presented, which show that an exposure of infested seed to sun for 9 hours during the hottest part of the year (May) may not be completely effective if the day, although otherwise clear, follows a shower of rain giving rise to cool breeze. On the other hand, under ideal conditions of heat transmission only half an hour's exposure of infested seed to sun during a comparatively cool month of March may give cent per cent mortality of larvae. At any rate, the factors on which the success of sun heat as a control measure depends are so variable that every individual exposure must be followed with an actual test of mortality among larvae inside the treated seed before the particular consignment can be said to have been effectively treated.

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THE LIFE-HISTORY, BIOLOGY AND ECOLOGY OF THE APPLE ROOT BORER, *LOPHOSTERNUS HUGELII* REDTEMBACH, IN KUMAUN

BY

R. N. SINGH, L.Ag., Assoc. I. A. R. I.

Government Fruit Research Station, Chaubattia, United Provinces

(Received for publication on 31st October 1941)

(With Plate LXVI)

LOPHOSTERNUS HUGELII Redtembach is an important pest of apple tree (*Pyrus malus*) in Kumaun. The larva is a long, thick, yellowish white grub. It attacks the roots and occasionally the portion of stem that remains in the ground. It feeds either by boring or by girdling around them. In most cases all the main roots are severed from the base and consequently the tree dies.

It has not been recorded as a pest of fruit trees in Kashmir, Baluchistan and Assam*. It also does not appear to have been recorded as such in any other country. Fletcher [1917] has given a brief account of its attack on apple trunks and roots in Kumaun. Beeson [1919] has mentioned *Quercus ilex* and *Quercus incana* as its food plants. In Kumaun, the author has observed the larva feeding mostly on dead stumps and roots of *Quercus incana* and on both living and dead roots of apple trees. Occasionally, it has also been found on dead roots of other forest trees and shrubs, such as *Pinus longifolia*, *Rhododendron arboreum*, *Pieris ovalifolia* and *Rubus flavus*. Among the fruit trees other than apple, the roots of pear (*Pyrus pashia*), peach (*Prunus persica*), cherry (*Prunus puddum*), and apricot (*Prunus armeniaca*) have also very rarely shown signs of damage by this borer, but in confinement it feeds on roots of all fruit trees grown in Kumaun hills. The details of the life-history of the insect has not been worked out so far. Stebbing [1914] seems to have taken some other larva for *L. hugelii*, as was also pointed out by Beeson [1919]. The account of its life-history as given by him has not been confirmed by the writer. The beetle was originally described in 1848 and re-described by Gahan [1906]. A technical description of the larva is given by Beeson [1919] to which some further descriptive notes are added by Gardner [1927].

Excepting these few details, no extensive work has been done anywhere either on its life-history or biology. A systematic work was, therefore, undertaken at this Station to study these points in detail. The work had to be spread over a number of years as the usual life cycle of the borer occupies four years. In addition to this, responses of the newly hatched and grown up borers to different environmental conditions have been studied with a view to devise control measures. Experiments on large scale were carried out under laboratory conditions for three consecutive years to see the effect of various soils and moisture contents upon oviposition response, development of egg and

* Private communications from the Directors of Agriculture

survival period of young grubs. Observations have also been made on the food materials of the grub, its habits inside ground and the effect of cultural operations on the development of egg and newly hatched grub.

Distribution in northern India

Gahan [1906] recorded it from Kashmir, N.-W. Province, Punjab and Assam. Beeson [1919] has in this connection mentioned Bashhar State, Mussoorie, W. Almora, Naini Tal and Siwaliks. It is very common in almost every orchard in Almora and Naini Tal districts, mostly on apple roots and dead oak stumps.

Economic status of the pest in Kumaun

It is a very serious pest of apple trees in these hills. As will be seen from the account given below, hundreds of apple trees are killed and thousands are rendered more or less unfit for bearing every year. In some portions of the orchards, the trees attacked may exceed 40 per cent. The amount of damage caused by this pest, recorded for a portion each of two orchards at Ramgarh and Chaubattia for the year 1931 and 1937 respectively, is given in Table I.

Table I

Number of apple trees attacked and killed by the apple root-borer

Name of orchard	No. of trees examined	No. attacked	Per cent attacked	No. killed	Per cent killed	Year of examination
Portion of an orchard at Ramgarh	1,081	451	41·7	59	5·36	1931
Portion of Govt. orchard, Chaubattia	488	82	16·8	28	5·74	1937

In every orchard certain portions are more susceptible to borer attack than the rest. Apparently the trees on dry sandy soil with little or no humus suffer most. Such soils are generally met with on ridges and steep slopes. Number of apple trees found attacked and those that were actually killed in different portions of the Government Orchard, Chaubattia, in the year 1936-37 are given in Table II. The orchard is situated on the Ranikhet, Chaubattia hill, at an elevation of 6,100—6,700 feet and is surrounded on all sides by oak and pine forests. There are hundreds of dead oak stumps all over the orchard and these afford a very suitable breeding place for the pest.

It will be seen from this table that the intensity of attack varies very much in different portions of the orchard.

TABLE II

Percentage of apple trees attacked and killed by the borer during the year ending 31 March 1937 in the Government Orchard, Chaubattia (in different blocks)

Blocks No.	Soil profiles			Percentage of trees attacked	Percentage of trees killed
	Sandy	Clayey	Sandy loam		
A	16	6	5	16·20	5·74
B	12	7	1	13·29	2·11
C	3	5	4	3·47	1·04
E	6	5	7	3·33	0·91
F	3	2	6	2·11	1·41
G	3	4	8	2·21	1·33
H	8	9	10	4·71	0·72
I	4	4	7	5·71	3·19
J	8	6	6	3·83	0·91
K	5	2	2	1·15	0·38
M	4	4	2	0·28	0·28
N	7	12	6	1·40	0·70
O	1	1	2	1·25	0·23
Q	4	6	13	2·22	..
R	2	10	12	0·58	..
S	16	14	9	6·23	0·74

Effect of the borer attack on apple trees

All the trees attacked by the borer are not affected to the same extent. Young tree having only one main root is killed outright, but a big, vigorously growing tree having many thick roots may not feel the attack to start with, provided the borer attacks only one root and confines itself to it. Young and small trees suffer most. Such trees will either die or remain very unhealthy, being suported by a few rootlets which are generally given out from the base when the main roots are cut off. Age, height and condition of all the attacked trees in the Chaubattia orchard were noted in the year 1934-35. The results summarized in Table III very clearly indicate that there is comparatively greater mortality in young and small trees.

TABLE III

Effect of the borer attack on apple trees of different ages and sizes

Condition of attacked trees	No. of trees	Average age in years	Average height in feet
Apparently unaffected . . .	18	11·45	5·3
Slightly affected	24	9·96	4·2
Affected	44	10·4	3·84
Severely affected	46	9·6	3·44
Dead	34	7·3	3·7
Total .	166		

DESCRIPTION OF DIFFERENT STAGES OF THE INSECT

As has already been pointed out, the adult and full-grown larvae have been described by Gahan and Beeson respectively.

The larva has been observed here from the time of hatching till pupation, *i.e.* for about three years and nine months. The changes occurring in its development at particular times are briefly described in this paper.

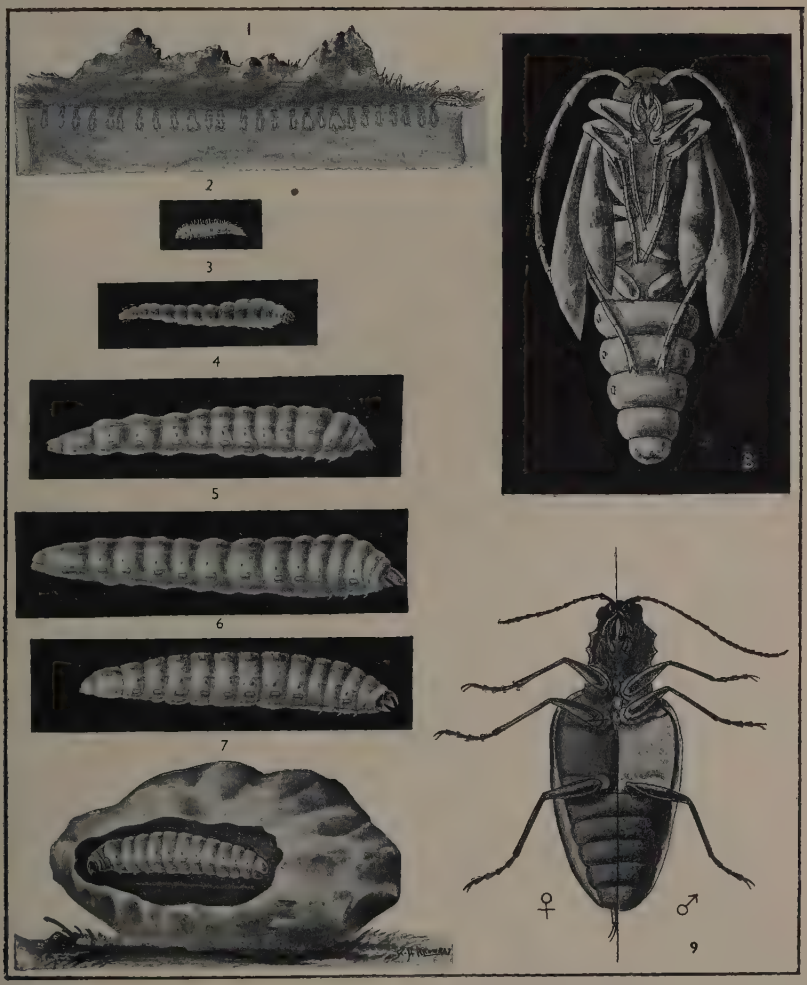
Egg

The eggs (Plate LXVI, fig. 1) are elongate-ovoid, yellowish white, measuring from 2·8 mm. to 3·2 mm. in length and from 0·8 mm. to 1 mm. in breadth. One end is slightly pointed and the other, which breaks open at the time of hatching, rounded. They are hard to withstand ordinary pressure and can easily be transferred from one soil to another several times by means of a camel hair brush. Irregular parallel ridges run longitudinally all over the outer surface and these under high power appear dotted. They cross one another at several places to form hexagonl areas of different sizes.

Different larval stages

The newly hatched larva is creamy white, thickset near the thorax ; light brown erect hairs all over the body. Length of an average-sized larva 3·5 mm., thickness 1 mm. near the thorax. In about four months it becomes 6—12 mm. long and body colour changes to reddish white. There are vast differences between the sizes of the different larvae of the same age. Thus the lengths of different larvae vary from 8 mm. to 15 mm. in the seventh month and from 12 mm. to 24 mm. in the ninth month. Up to about ten months after hatching the body colour remains reddish white, but after that the reddish tinge gradually disappears. In the 14th month, when the lengths of different larvae vary from 18 mm. to 31 mm., the general body colour becomes dark brown, but the ridges of every segment remain white. There is no marked difference in

Lophosternus hugelii



1. Eggs laid in the ground ($\times\frac{1}{4}$); 2. Newly hatched grub (nat. size); 3. One year old grub ($\times\frac{3}{8}$); 4. Two-year old grub ($\times\frac{5}{16}$); 5. Three-year old grub ($\times\frac{5}{16}$); 6. $3\frac{1}{2}$-year old grub ($\times\frac{5}{16}$); 7. Grub inside pupating chamber ($\times\frac{1}{2}$); 8. Pupa ($\times\frac{3}{4}$); 9. Ventral view of both sexes ($\times\frac{1}{2}$)

the body colour in the following 11 months, but the larva goes on growing all through the period. In the end of the second year, larvae are from 74 mm. to 100 mm. long. There is very slight increase in the body after this period, but the body colour gradually changes to yellowish white. In the beginning of the fourth year the body begins to contract and yellowish tinge goes on deepening till 42nd month after hatching when the borer leaves the apple root and makes an earthen cell in the ground near the root to pupate inside it. Inside this earthen cocoon there is no change outwardly in the body for the first three months except that the body goes on contracting and a watery fluid is secreted all the time, and this makes the inner surface of the earthen cocoon very clean and uniform. At the end of these three months, the grub is only about 38 mm. long with a transverse orange-red line on its head. Actual pupation begins after this, i.e. in the end of May and adult may emerge any time in June and July after the first shower of rain (Plate LXVI, figs. 2-9).

Technical description of the mature larva

Beeson [1919] has described the larva and Gardner [1927] provides some additional information. The following summary is given to identify the larva.

Body straight, more or less cylindrical, tapering gradually posteriorly and gradually enlarged in the thoracic region. Head large, deeply embedded in thorax, depressed. Labrum free and distinct. Ventral mouthparts not retracted, the submentum not continuous with prothoracic skin (i.e. the anterior ventral margin of the head capsule is visible). The dorsal surface of prothorax with a large transverse trapezoidal area and quite different in appearance from the ventral surface. Spiracles with oval peritrames (family characters).

The ventral surface of the head with a smaller anterior foramen in addition to a larger posterior foramen (in other subfamilies there is no anterior foramen). Mandibles with the distal cutting edge oblique, the lower extremity more acute (distinction from Cerambycinae). Head only slightly longer than wide, emarginate posteriorly. Legs small but quite distinct. Dorsal ambulatory areas of abdomen with two, the ventral with one, transverse impressed lines. Caudal extremity without points or spines (sub-family characters).

Antennae with three segments, the apical one very small (note that a basal connecting membrane is not a segment). The anterior (epistomal) margin of the head forms a flat rather short projection, with angulate extremities, over the whole clypeus ; behind that is a moderately raised but distinct transverse ridge which is at most feebly depressed medially. The head has a quite strong posterior emargination. There are no distinct ocelli. The pronotum is irregularly rugulose (generic characters).

The larva is yellowish white with the exposed part of head and mandibles black, prothorax reddish anteriorly and laterally. The skin smooth with sparce and very inconspicuous hairs. Length up to about 106 mm.

The pupa

The pupae are of various sizes, measuring from 18 mm. to 31 mm. in length. It is of exarate type, the head strongly bent downwards and the abdomen sharply tapering towards the caudal end. From the time of the first appearance of the organs of locomotion, the colour gradually changes from yellowish white to chestnut red.

The adult (beetle)

Gahan [1906] has described *Lophosteruns* and has also given the main characters of *hugelii* to distinguish it from other species of the genus. The adult found and studied in Kumaun conforms to Gahan's description except in a few minor details like the variation in body lengths, etc. A brief description of the beetle, embodying its important generic and specific characters, is given below :

Male.—26—58 mm. long, chestnut red in colour, the head and prothorax darker than elytra. Head elongated behind the eyes, short in front, strongly and closely punctured. Mandibles long, curved downwards, crossing when closed, their inner edges sharp. Palpi long, last joint gradually widened towards the extremity. Antennae a little shorter than the body, 11-jointed, first joint not reaching beyond the hind margin of the eye, third to tenth acutely produced at the apex on the anterior side, third with sharp anterior edge near which it is finely and very closely puntualate. Prothorax transverse, convex above, its lateral edge oblique and denticulate in front, produced into a spine at the middle into another spine near the front margin and sharply angulated near the base. Pronotum finely and closely punctured in front and for some distance back along each side of the middle line, more strongly punctured towards the sides, its hind angles more or less obtuse. Elytra more than twice as long as broad, slightly narrowed behind, more or less round at the apex, rugulose, the ridges finely punctured ; each with two or three feebly raised obtuse costae. Hind breast covered with a tawny coloured silkly pubescence. Legs long, tarsi elongated, the first joint as long as the second and third together, third joint bilobed, the lobes rounded at the end. Last ventral segment sinuate at the apex.

Female.—Head and mandibles shorter. Antennae hardly reaching to the middle of the elytra, more slender and less strongly serrate than in the male, the joints from the fifth only, acutely angulate at the apex. Hind breast bare of pubescence. Last ventral segment with rounded hind margin.

Out of the 100 males the average weight of the ten biggest ones was 3·75 gm. and that of the ten smallest ones 0·62 gm., i.e. the biggest beetles are about six times heavier than the smallest ones. There is similar variation among the female beetles also.

Life-history

Emergence

Emergence of adults from the soil starts at the break of monsoon about the end of June and lasts for about a month. Early showers during the first week of June, preceded by hot, sunny days, bring forth some beetles earlier, but occasional rains in April and May and low temperature in June delay emergence. In exceptional cases when the temperature in the summer months remains below normal throughout with frequent showers of rain, the pupation period is very much prolonged. As a result of this only a few beetles emerge. Such a case was observed in 1936. Beetles of both sexes are attracted to light, but collections at light contained mostly males. Out of 224 beetles collected, only 14, i.e. 6·25 per cent of the total were females.

Habits of the beetle

During the day time beetles have never been seen on the wing. In the orchard they remain motionless under grass in dark holes. Inside the cages, if there be no grass, they make small holes in the soil along the sides of the cages just enough to hold their bodies. They do not move about during the day unless disturbed and then they search their mate and begin to copulate. The female goes on depositing eggs while moving. They move about freely during night and fly to the nearest strong light. Beetles do not feed and they are short lived. Copulation and egg-laying begin soon after emergence. The male dies only a few days after, while the female dies after depositing all its eggs.

Oviposition

Soon after emerging from the ground the beetles seek their mates. The male follows a female and rides over her, in most cases from behind but occasionally from the sides also. Holding the female by its forelegs at the hind margin of its prothorax, it extends its pygidium and inserts the aedeagus into the female's genital aperture. The female may remain stationary while the copulation is going on or move about with the male on its back. The male, throughout the process, moves its legs backwards and forwards. The female may copulate several times with the same or different males every day for the whole of its life of about 16 days. Beetles can be seen copulating at any time during the night, but during the day they seek mate only when disturbed from their hiding places.

The female starts egg-laying a few hours after copulation and goes on copulating and laying eggs daily for the whole of its short life. When about to lay eggs, she moves about on the ground in search of suitable place, dragging behind her extended ovipositor. On finding a desired place, she stops and thrusts her ovipositor into the soil and deposits one or more eggs to a depth of about 8 mm. She then moves on to some other place and lays eggs similarly. Unlike many other insects, the female does not lay eggs particularly on or near the food material of the young larva. Generally, eggs are laid singly, but batches of two, three and four eggs have also been found. When more than one egg is laid at the same time, these remain attached to one another longitudinally by means of a gelatinous substance. Very often small particles of soil are found adhered to the eggs.

Observations made on the egg-laying capacity of the female and the duration of egg-laying period have shown that the average number of eggs laid by one female is about 300 and the average egg-laying period 16 days.

Results of the experiments on the effect of various soils and moisture contents upon oviposition response carried out at this station have indicated that 20—40 per cent saturation of soil is most suitable for egg-laying in all the three kinds of soils, viz. clayey, sandy and organic and that sandy soil is preferred by the beetle for this purpose.

Hatching

Laboratory experiments carried out to study the different aspects of hatching showed that in normal cases about 80 per cent of the eggs hatch.

The hatching continues for about 40 days, but about 70 per cent of the eggs hatch within 29 days. There is, however, a definite correlation between the moisture content of the soil and hatching. The most suitable saturation for hatching was found to be 20—40 per cent. Below and above this range of saturation, the percentage of hatch goes on decreasing.

Habits of the grub

After hatching young grubs move about on the ground at random. Observations showed that they do not possess food direction sense and hence very few of them succeed in reaching the roots. The newly hatched grub can live in any soil with or without any root for over 20 days provided the soil contains the right amount of moisture (20—40 per cent saturation). If during this period young larva happens to come in contact with some root, it attaks it ; otherwise it dies in the soil. It does not seem to feed on roots daily, for, at times it has been observed lying in the soil near the root for several days. When a root has all been eaten away, the grown-up borer moves about very slowly inside the ground, not necessarily in the direction of another root. It has been observed to survive in orchard soil without roots for about three months and, if within this period a suitable root is reached, it is attacked but, unless such root is very near, it is very likely that the borer after leaving a root dies before it can get on to another. Of the grubs hatching out every year, a few go on feeding on roots for about 3½ years, after which they leave the roots and prepare pupating chambers of particles of soils near the attacked roots.

Of the 46 borers found on or near the apple roots, 45 were within 3—10 in. depth and 77 per cent of these were confined between 4—8 in. As regards the distance from the base of the tree, one was found 30 in. away and the rest were all within 12 in. of it ; 56 per cent of the total number were found exactly at the base.

Observations made on the effect of different soils and moisture contents on survival period of young grubs have indicated that in all soils the mortality of grub is accelerated by the increase of moisture above 40 per cent saturation.

From a review of the results of the three years' (1937-39) observations on the food material of young grubs, it may be concluded that young grubs after hatching feed on all kinds of organic matter present in the soil, but farmyard manure, leaf-mould and well-decomposed orchard vegetation shorten their lives as they can live in soil, practically free from organic matter, for a longer period.

Pupation

Pupation invariably takes place inside the earth cell prepared by the grub.

First of all the body contracts and there is some watery secretion. The head then slightly bends downwards and faint red lines gradually appear on the thorax. After about three months' rest inside the cell the legs, antennae and wing-pads begin to appear. The final stage is reached about the middle of June in normal years, but the beetle does not emerge from the ground before the first heavy rainfall.

Survival of the borer to maturity

Under artificial conditions very few borers were reared to maturity. Out of 455 newly hatched grubs kept in breeding cages in 1932, only one survived for about 3½ years and died just when it had started pupating, and of the 660 kept under similar conditions in 1935, only five survived till March 1938. One of these assumed adult stage in July 1938 and three in July 1939. The remaining one died sometime before November 1938. Observations have been made on various occasions on the survival of the grubs in nature and every time it was found that most of them died before reaching maturity. One female beetle lays 300 eggs on average and it has been observed for several years that the percentage of trees attacked by the borer remains almost constant in every orchard in Kumaun. If it is assumed that equal number of males and females are produced in nature, it means that only two out of 300, i.e. 0·6 per cent reach maturity.

EFFECT OF MOISTURE ON THE NEWLY HATCHED GRUB

Observations on the effect of different soil saturations on the survival of the grub have indicated that the young borer thrives best in soils with 20—40 per cent moisture contents. It does not do well in very wet soils. The present experiment was carried out to see how long the borer can live in dry soil. Six newly hatched grubs were liberated on each of the various sorts of dry and moist soils and their survival period noted. The results are briefly summarized in Table IV.

TABLE IV

Effect of moisture on newly hatched borers

Name of material	Date and time of liberation	Date and time of dying	Average survival period
Moist garden soil (20—40 per cent saturation)	27-7-35 10 A.M.	19-8-35	23 days
Sun-dried garden soil	27-7-35 10 A.M.	27-7-35	7 hours
Moist subsoil (20—40 per cent saturation) .	13-8-35 10 A.M.	5-9-35	23 days
Sundried subsoil	13-8-35 10 A.M.	13-8-35 4-30 P.M.	6½ hours

It is clear from Table IV that moisture in the soil is necessary for the life of the young borer.

Effect of temporary shortage of soil moisture

The development of eggs

To determine the effect of temporary drought on hatching of eggs, six eggs of different ages were kept in each of the ten glass dishes containing sun-dried soil on 27th July 1935. The soils of five dishes were moistened to about 30 per cent saturation after two days and those of the remaining after five days. The number of larvae hatching out in every dish was noted down. The results are tabulated in Table V.

Table V

Effect of temporary shortage of soil moisture upon the development of eggs of different ages

Cage No.	Number of eggs	Age of eggs	Time of their remaining in sun-dried soil	Average No. of days taken in hatching	Number hatched	Percentage of hatching
1 . . .	6	Fresh .	2 days .	26 days .	3	50
2 . . .	6	6 days	,, .	33 ,, .	4	67
3 . . .	6	12 ,,	,, .	No hatching	..	0
4 . . .	6	18 ,,	,, .	36 days .	6	100
5 . . .	6	23 ,,	,, .	28 ,, .	6	100
1 (*a*) . . .	6	Fresh .	5 days .	26 ,, .	3	50
2 (*a*) . . .	6	6 days	,, .	34 ,, .	4	67
3 (*a*) . . .	6	12 ,,	,, .	No hatching	..	0
4 (*a*) . . .	6	18 ,,	,, .	30 days .	6	100
5 (*a*) . . .	6	23 ,,	,, .	28 ,, .	6	100

Although there were only six eggs in each dish, it will be quite fair to draw certain conclusions from this experiment as the results obtained are absolutely similar in both cases. It is clear that temporary drought on or about twelfth day after laying is fatal, but it does not seem to affect the younger and older ones much.

The experiment was repeated in 1937 and the data, which are briefly expressed in Table VI confirmed the results of the previous observations.

TABLE VI

Effects of temporary shortage of soil moisture on the development of eggs

Serial No.	Age of eggs on 19-7-35	No. liberated	Time of their remaining in sun-dried soil	Condition of eggs on 22-7-37	Number hatched
1	Newly laid .	23	19 to 22 of July	Looking healthy	23
2	8 days old .	16	Do. .	Shrivelling .	1
3	14 „ „ .	9	Do. .	Do. .	Nil
4	21 „ - „ .	11	Do. .	All eggs hatched	11

Newly hatched grub

The object of the experiment was to see if a temporary drought affects the young borer grub ; for, an occasional dry spell during rainy season is not uncommon. Four cages were prepared, two with a 2-in. layer of moist soil (20—40 per cent saturation) at the bottom and 2-in. layer of sun-dried soil at the top. The other two with the dry layer of soil at the bottom and the moist layer of soil at the top. Twenty young grubs were liberated in each cage at all the four different positions.

The results, summarized in the appendix, do not show any indication of the grubs moving in any particular direction. Some of them by chance happen to reach moist soil below, but quite a few come to the surface in dry soil and die.

EFFECT OF DIGGING THE GROUND ON THE EGG AND NEWLY HATCHED GRUB

Eggs are only slightly affected by the disturbance in the soil caused by cultural operations such as digging and hoeing. A piece of ground in which hundreds of eggs were laid was dug up to expose the eggs to the surface but only one egg was exposed. Disturbing the position of the egg does not affect its development so long as it remains in moist soil. In the laboratory thousands of eggs have been removed from the soil and transferred to various cages but hardly any of them was injured. An attempt was also made to scrape off an uniform layer of about ½-in. from the upper surface of the ground so that all the eggs could be removed from the ground and destroyed. But as in the rainy season the ground remains wet and grass grows everywhere, such layer could not be scraped off. The egg-shell is hard to stand ordinary handling and shaking. In laboratory they were taken out from soil, counted, and replaced in the soil without least injury to any of them. All that was required for the development of eggs was that they should remain in moist soil at 78°—92°F. These observations show that digging or hoeing of the ground is ineffective for destroying the eggs and besides, eggs are also laid in banks which offer almost as much space for egg-laying as the terraces which for other reasons should not be disturbed.

A piece of land, about 50 sq. yards in area, containing hundreds of young grubs was dug up but not a single grub was exposed to the surface. One hundred grubs, which were liberated in a space of two square yards of dug-up area, entered the ground in one hour and 20 minutes, but when liberated on hard, undug, practically dry land, they could not enter the ground and were taken away by ants. Digging and hoeing disturb their positions in the soil, but they still remain covered with moist soil in most cases which is the only requirement to keep them alive. Very few are actually injured or killed with the implements at the time of digging. These observations show that digging is ineffective for destroying the young grubs present in the ground.

Movements of the grub in the soil

(*a*) Four glass cages with moist soil and bits of roots buried in it were kept in the laboratory and two grubs were liberated in each of them. It was observed that the grubs moved about at random inside the soil, sometimes going downwards and returning upwards or going towards the sides of the cages. Very often they were seen making rounds in the same direction over and over again. For several days they went on moving about in the soil without coming in contact with the food material which was placed very close to them. Sometimes they happened to reach within a quarter of an inch of the food material yet they did not seem to realize it and came back. Out of eight grubs only one found the roots within 30 hours, three between 9 and 13 days, and the rest died without being able to reach the roots.

(*b*) One hundred young grubs were liberated in a cage at a distance of three feet from the roots buried in the same cage. On examination of the roots after four months, only seven grubs were found to have attacked the roots, showing that only a small percentage can reach the roots if they hatch about three feet away from them.

(*c*) Out of 220 grubs liberated on the roots of four apple trees only three could attack the roots.

These observations indicate that the newly hatched grub has no food direction sense.

Observations on the movements of grown-up borers also lead to the same conclusion. The movements of four grubs were traced for eight months. None moved in any particular direction and the average monthly movement was about four inches in a month. The movements of three grubs, liberated in the ground near an apple tree at one foot, three feet and five feet away from it, were traced for two months. Only one of them, which was liberated one foot away from the base of the tree, succeeded in reaching the roots. The movements of any of them were not necessarily towards the roots. These observations also showed that after leaving a root, the borer often remains at the same place for over a month. It does not move in any particular direction and often returns to the same spot from where it had started. It moves about at random and in a very zigzag way, mostly remaining within a few inches of its starting place.

Nature and mode of injury

Examination of the borer-attacked roots of 155 apple trees at Chaubattia in 1935 has shown that it feeds only on thick portions of roots. The

rootlets and the ends of main roots remain in the ground, dislocated. The minimum thickness of a root on which a borer was observed feeding was 0·83 in. in diameter. In most cases the attacked root is cut off from the base. Out of 155 roots attacked, 144 were completely severed from the bases of the trees. In 52 out of 155 cases the root was found severed from the base but there was no borer feeding on it. It had either disappeared or had started feeding on another root close to it. In other cases the borer attacked a root near the base of a tree and followed it on towards its tip. The root was either bored or girdled if it was not thick enough to hold the borer. In 94 per cent cases, the root was attacked just at the base of the tree. In other cases, the borer generally started feeding from within a distance of four inches from the base. The depth at which the attack started varied from 2 and 11 inches but in 95 per cent cases borer started feeding between 4 and 8 inches depth.

Alternative food material of the borer

In nature large borer grubs identified as *Lophosternus hugelii* Red. have been found feeding mostly on the roots of apple and dead stumps of oak. Very rarely roots of walnut, pear, peach and cherry have shown signs of damage by this borer. Pieces of root of all the fruit trees grown in Chaubattia orchard and of oak, rhododendron and pine were buried in moist soils in separate cages in August 1935 and 60 young grubs were liberated in each cage in the same month. Roots of all the cages were examined in December of the same year. Percentage survival of grubs in different cages was : apple 38, walnut 32, pear 15, peach 50, plum 17, cherry 20, apricot 45, nectarine 23, oak 18, pine 25, and rhododendron 23.

Observations were also made on the food material of grown-up borer. Roots of apple, pear, peach, plum, apricot, chestnut and walnut were buried separately in soil and five grown-up borers were liberated on each on June 6, 1936. A similar set of cages was arranged two months later and borers were similarly liberated. During the first few months they fed on all the roots and a few lived in every cage till September 1938. After that all the grubs died in cages containing cherry roots, but lived on all other roots till January 1937. Between January and June of the same year, all borers died on pear roots and a few months later on apricot roots also. In August 1938, living borers were found only on apple and chestnut roots. After this all the borers except one on chestnut roots gradually died. This one had made a pupating chamber in February 1939, but died while still in pupal stage. These observations show that the borer is likely to feed on roots of all the fruit trees grown in Kumaun hills when forced to do so.

Summary and conclusions

Lophosternus hugelii Red. is a very serious pest of apple trees in Almora and Naini Tal districts, where in certain portions of every orchard about 40 per cent of the trees are attacked. Very few of the attacked trees survive and bear normal crop. It is recorded from all the hills in northern India up to a height of about 7,000 feet and also from Siwaliks. It attacks both living and dead apple roots and is found mostly on underground portions of dead oak

stumps. Fruit trees other than apple rarely show signs of damage by this borer.

All the stages of the insect have been described.

Beetles emerge from the ground just after the break of monsoon and mating and egg-laying take place soon after emergence. Eggs are laid in the ground to a depth of about 8 mm. and most of the eggs hatch in 24-29 days. One female lays on an average about 300 eggs. After hatching the young grubs move about in the ground at random as they do not possess any food direction sense. They attack apple roots if by chance they come across them. They go on feeding on roots of apple and dead stumps of oak for about 3½ years, after which they leave them, make small chambers of earth and retire in them to pupate. Normally the adult stage is reached by the middle of June and beetles emerge after the first heavy shower in the end of June or beginning of July.

The results of the experiments carried out to see the effect of different soils and moisture contents upon oviposition response, development of egg and survival of young grubs have definitely indicated that 20—40 per cent saturation of soil is most suitable for egg laying and development of egg in all the three kinds of soils, viz. clayey, sandy and organic, and that sandy soil is preferred by the beetle for depositing eggs. In all soils mortality of the grubs is accelerated by the increase of moisture above 40 per cent saturation. In nature large borer grubs have been found feeding mostly on roots of apple and dead stumps of oak. Roots of walnut, pear, peach and cherry have very rarely shown signs of damage by this borer but in confinement it feeds on roots of all the fruit trees grown in Kumaun hills. Temporary shortage of soil moisture below 20 per cent saturation on or about 12th day after laying is fatal to eggs but does not seem to affect much the younger and older ones. In dry soils young grubs cannot live for more than seven hours but in moist soil with only a trace of organic matter in it, they survive for about a month. If the upper few inches of ground containing young grubs gets dry, some of the young grubs by chance reach the moist soil below but quite a few come to the surface in dry soil and die. Digging or hoeing of the ground does not effect any substantial reduction in the number of eggs and newly hatched grubs as the egg-shell is hard enough to withstand the ordinary sifting of soil and young grubs re-enter the soil if exposed to the surface. In captivity borer feeds on roots of all kinds of fruit trees grown in Kumaun hills and of oak, pine ad rhododendron. The first attack of the borer on apple root starts near the base of the tree at a depth of about 4—8 in. It attacks the thick roots only, rootlets and ends of the main roots remain in the ground, dislocated from main roots. After leaving a root borer moves about very slowly, generally, at the rate of about 4 in. in a month, and not necessarily in the direction of another root. Grown-up borer can survive in soil without any root for about three months. If during this period it comes in contact with some root, it attacks it, otherwise it dies inside the ground. The chances of a borer attacking another root after leaving one are, therefore, very rare.

Acknowledgements

Observations on the life-history were made in private orchards at Ramgarh and thanks are due to the owners for allowing the examination of the roots of

attacked trees. The rest of the work, which formed a part of the programme of the Entomological Section of the Hill Fruit Research Scheme, financed by the Imperial Council of Agricultural Research, was done at Chaubattia under the supervision of Messrs P. B. Richards, and H. N. Sharma, Entomologists to Government, United Provinces. The financial help of the said Council is gratefully acknowledged. Thanks are also due to Mr J. C. M. Gardner, Systematic Entomologist, Forest Research Institute, Dehra Dun, for describing the mature larva and to Dr U. S. Sharga, Entomologist to Government, United Provinces, for making certain valuable suggestions.

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APPENDIX

Effect of temporary shortage of soil moisture upon newly hatched grubs

Cage No.	Description of cages	No. of grubs liberated	Where liberated	Observations		
				One hour later	Two hours later	24 hours later
I	2 in. moist soil above 2 in. dry soil below	20	In dry soil	8 seen moving about at random in dry soil	One has gone to moist soil	10 found in moist soil, 4 in dry soil which had become partially moist by that time and 6 were found dead in dry soil
II	2 in. dry soil above 2 in. moist soil below	20	In moist soil	4 moving in dry soil and the rest in moist soil	None visible, traces of their movements in both dry and moist soils	Grubs had gone up to 1½ in. in dry soil. 6 were found in dry soil which had absorbed moisture by that time, 12 in moist soil and 2 found dead in dry soil
I (*a*)	2 in. moist soil above 2 in. dry soil below	20	In moist soil	None has gone to dry soil	There are traces of their movements in dry soil	10 found in moist soil and 10 in dry (partially moist) soil. All living
II (*b*)	2 in. dry soil above 2 in. moist soil below	20	In dry soil	4 have come out on the surface and 8 have gone down in moist soil	3 on the surface, 1 entered the soil, rest not visible	10 in moist soil, 8 in dry (partially moist) soil and 2 found dead on surface

STUDIES ON KUMAUN HILL SOILS

II. EFFECT OF TERRACING AND CULTIVATION ON SOIL TYPES AT CHAUBATTIA*

BY

B. K. MUKERJI, Ph.D., D.Sc.

Agricultural Chemist to Government, United Provinces

AND

N. K. DAS, M.Sc., Assoc. I.A.R.I.

Research Assistant Soil Chemist

(Received for publication on 9 October 1940)

THE soil formations under unterraced, i.e. natural conditions, together with the geology, vegetation and climate of Chaubattia have already been reported [Mukerji and Das, 1940]. The climate of this station approximates to that of humid temperate zones. The parent rock materials are mostly biotite-schists and granite-gneiss. At some places both granite and biotite appear to be present intimately mixed with each other. The common vegetation all over these hills is typical of the forest flora generally found in the humid temperate zones. As already indicated [Mukerji and Das, 1940] four major genetic types of soil formation occur on this orchard. These are brown forest soils, podsols, red loams, and wiesenboden, of which brown forest soils, constituting the greater part of the orchard is by far the most important for fruits like apples, pears, peaches, plums, apricots, etc.

One of the most important pedogenic features observed previously is that there is under natural conditions a marked tendency for the brown forest soils to assume certain characteristics of podsol formations. The present paper has been written with the main object of showing how the usual practice of land utilization in these hills by terracing and cultivation on terraces affects the natural dynamical processes of soil formations in this locality. In view of the importance of brown forest soils to fruit growing in these hills, attention has in the present instance been wholly directed to the pedological changes in relation to this genetic type and its variants.

The Chaubattia orchard has a northern aspect, which is the best for growing temperate fruits, with an elevation of 6,100 to 6,900 feet above sea-level. The total area of the orchard is 158·61 acres of which about 100 acres are laid out with blocks of all temperate fruits and the remaining portion is under forest. Out of the 100 acres under fruits, an area of about 85 acres is terraced and the remaining only 15 acres is under contour plantation. A

* The researches forming the subject matter of this publication were carried out in the Soil Chemistry Section at Chaubattia (Kumaun Hills) under the Hill Fruit Research Scheme, financed partly by the Imperial Council of Agricultural Research (India)

detailed study of the effect of terracing on the natural soil types of this station constitutes, therefore, a very important item in the programme of the soil work in progress.

Apart from all other considerations, the successful function of terracing lies in the fact that it results in checking soil erosion along slopes on one hand, and conserving subsoil moisture on the other. The usual bench type of terraces which are suitable for the hill conditions does check erosion to a considerable extent ; but for moisture conservation these do not appear to be effective, particularly along very steep gradients. Along steep slopes the subsoil remains immature and sandy. The detailed soil survey of the orchard shows that subsoil texture and therefore moisture conservation in the subsoil regions are dependent on the slope conditions. It will be clear from Table I in which the distribution of 716 profiles according to slope gradients has been shown that sandy profiles are mostly situated along slope gradients of 45° and more.

TABLE I

Textural characters of soil profiles along different slope gradients

Serial No.	Textural characters of profiles	Total number	Distribution of profiles according to slope gradients in degrees						
			10°	20°	30°	45°	50°	60°	More than 60°
1	Stony and sandy . .	378	1	5	8	68	89	143	64
2	Loamy	153	2	6	12	51	41	37	4
3	Clayey	185	9	18	52	77	21	7	1

LITERATURE

The scientific study of soils particularly in Europe and America has now definitely established the nature and properties of the various genetic types. The possibilities of this method of soil classification from the point of view of land utilization for agricultural purposes are being thoroughly investigated by the soil scientists all over the world. Reference in this connection may be made to the works of Norton [1939], Kellog [1937] and de Sigmond [1932]. The clear recognition of the dynamic reactions of the soil types to the different cultural operations necessary for agronomic utilization has opened up a very profitable field of research in which much fundamental work yet remains to be done.

Brown forest soils or the so-called brown earths have not been assigned a definite place in the International classification of soils. Some have considered brown forest soils to be a definite soil type ; whereas, others would like to call these ' concealed podsols ' in which the podsolic processes in progress have not fully expressed themselves. This confusion is due mainly to the fact that sufficient account has not been taken in every case of the environments of the brown forest soil studied.

It appears from the investigation of Muller [1887] on Danish heaths that brown earths due to a change in vegetation factor can slowly change into

podsols. Tamm [1932], however, clearly postulates the view that 'there is no general tendency, as some believe, towards the podsolization of brown forest soils. The reverse is much in evidence' and that 'the brown forest soils as a climatic type are very apt to persist; they belong to the medium humid temperate climate where deciduous forests represent the natural vegetation'. Balleneggar [Joffe, 1936] has found the soil from ploughed field to be more podsolized than one from beach forest. In dealing with the incipient podsolic processes in brown earths, Jack [1934, 1] says: 'The process is one of simple solution by carbonated rain water, and goes on continuously in the humid brown earth climate. There should therefore be a tendency towards increasing soil acidity, and if this happens, the vegetation may ultimately change from deciduous to coniferous forest and podsolization will occur. But if the vegetation can absorb sufficient bases and return them in the humus sufficiently quickly for the soil to counteract the leaching effect, the brown earth and its corresponding vegetation form a stable system which is only disturbed by the slow wastage that accompanies every natural process'. The following has also been taken from the work of the above author [Jack, 1934, 2]: 'An interesting phenomenon sometimes arises when birch replaces beech or coniferous forest on podsolized soils. Birch produces a slightly acid well buffered type of humus, and in course of time may reverse the podsol process that went on under the earlier vegetation; the soil gradually develops the characteristics of a brown earth—a more fertile soil type than the podsol. This represents a kind of natural regeneration of the soil, and birch now holds a high place as a soil improver in the silviculture of many northern countries'. A review of all the available informations leads one to the conclusion that podsolic formation is a reversible process and this seems to have been confirmed by the studies reported in the present paper.

A number of terraced and cultivated soils has been studied by Shaw [1932] in Eastern Central China. It appears from his descriptions that these soils possess certain brown earth characteristics.

Methods and procedure

Field survey

A detailed survey of the terraced soils of the Chaubattia orchard at 50 feet, both along and across the slopes, has been completed. The unit of our study was taken to be the soil profile. Pits were dug at the corresponding points of horizontal and vertical cross-lattices of the orchard map at regular distances of 50 feet apart. These pits were sufficiently broad for an observer to go inside and note visually the profile characteristics. In soils other than the clayey ones, digging of pits for soil survey was continued up to the partly decomposed parent material. In the case of clayey profiles, however, the pits were dug up to the impervious clay pan.

Observations in regard to the characteristics of each horizon, particularly colour, texture, structure, depth, hardness, concretions and cementations were made *in situ* and representative samples were obtained from each horizon for laboratory studies. Due chiefly to their positions along different slopes and aspects the soils under field conditions were found to have different moisture contents and, therefore, it was felt desirable to supplement field

observations on colour and texture with similar observations made in the laboratory under fairly uniform and controlled conditions. The soil samples obtained from the fields were therefore air-dried, and observations on colour and texture repeated under air-dried and moisture-saturated conditions. This undoubtedly afforded a fuller knowledge of the horizons than that based on observations noted in the field alone.

Analytical method

Air-dry sample was broken down with a wooden pestle and passed through 2 mm. sieve ; the materials remaining on the sieve were reported as stones and gravels. Two-millimetre samples were used for both mechanical and chemical analysis.

Mechanical analysis

Pretreatment and dispersion were effected according to the recommendations of the International Society of Soil Science, followed by pipette sampling for silt and clay.

Chemical analysis

Hydrochloric acid extract was prepared according to the directions of the British Agricultural Education Association [Wright, 1939]. Lime and magnesia were both determined titrometrically. Sesquioxides were precipitated by ammonium chloride and ammonia, and iron estimated by titration with standard potassium permanganate.

Organic carbon.—This was estimated by Walkley and Black's [1934] method with potassium dichromate and ferrous sulphate.

Organic nitrogen.—For the determination of total organic and ammoniacal nitrogen, the usual Kjeldahl's method was followed after pre-treating the soils as suggested by Bal [1925].

p*H*.—The quinhydrone electrode method was adopted for the determination of *p*H values with soil and double distilled conductivity water ; ratio 1: 2·5.

Exchangeable acidity and base exchange capacity. —These two were determined in the same sample by Parker's barium acetate and ammonium chloride method [Pierre and Scarseth, 1931], and percentage base saturation was calculated according to the formula given below :—

$$\text{Per cent base saturation} = 100 - \frac{\text{Exchangeable hydrogen} \times 100}{\text{Base-exchange capacity}}$$

Clay analysis.—Clay samples were collected according to the method suggested by Robinson [1932] and were analysed as a silicate after fusion with sodium carbonate.

Moisture.—10 gm. of air-dry soil were dried in an oven at 105°C till constant weight. The loss in weight was reported as hygroscopic moisture.

Loss on ignition.—The oven-dry soil was ignited with frequent stirring at slow red heat till constant. This operation takes usually six hours.

Data and discussion

In all 30 complete profiles from terraced areas scattered over the orchard have been analysed. The analytical data in regard to five typical profiles are given in Tables II—XI. The visual survey of 716 profiles from

all the terraced part of the orchard has shown that the soils which have in course of time consolidated possess certain characteristic features necessitating their classification under two main groups. Soil profiles examined on the terraces, prepared only recently, have not been included in our present studies, as the natural soil forming features have had no time yet to stabilize themselves on these soils.

BROWN FOREST SOILS

A large majority of the terraced soils so far studied by us can be classified as brown forest soils. The descriptions of three typical profiles, one from each of sandy, loamy and clayey textural groups, are given below :—

Name of the Profile	Texture of the Profile	Depths	Horizons	Descriptions
13 R 4 .	Sandy .	0—4 in. .	A .	Micaceous ; brownish grey ; sandy ; structureless and stony ; more grey when wet.
		4 in.—3 ft.	B	Brownish ; more brown when wet ; stony sandy ; more sandy than above but more compact. Structure not pronounced cloddy.
		3—5 ft.	C	Yellowish ; micaceous ; more yellow when wet ; sandy ; stony with no structure ; consistancy loose.
X18 Y 7 .	Loamy .	0—6 in. .	A	Greyish brown ; micaceous ; loamy with some stones ; more grey when wet ; angular cloddy with medium particles to big granules.
		6 in—1 ft. 6 in.	B	Brown ; more brown when wet ; silty loam ; more indurated than above ; medium to big clods.
		1 ft. 6 in.— 2 ft. 10 in.	C	Micaceous ; yellowish brown ; structureless ; brownish when wet ; sandy with very many stones.
15 R 3 .	Clayey .	0—5 in. .	A	Greyish brown ; stony loam ; more grey when wet. Finely granular to crumby with plenty of feeder roots.
		5 in.—1 ft. 6 in.	A2	Yellowish brown ; more brown when wet. Medium to fine clods ; clayey.
		1 ft. 6 in.— 2 ft. 8 in.	B	Brown ; more brown when wet. Big clods resembling more or less columns. Very clayey.
		2 ft. 8 in.— 3 ft. 6 in.	B+C	Brownish ; intense brown on wetting. Clayey with very many air holes ; prismatic with dark circular incrustations, and concretions.

The analytical data of the above profiles—chemical and mechanical—are given in Tables II—VII.

TABLE II

Analytical results of terraced brown earths—sandy soils

Chemical

Name of profile	Depth	Horizon	Determinations.										
			Moisture (per cent)	Loss on ignition (per cent)	Organic carbon (per cent)	Organic nitrogen (per cent)	C/N	pH	Insoluble matter in strong HCl (per cent)	Fe_2O_3 (per cent)	Al_2O_3 (per cent)	MgO (per cent)	CaO (per cent)
13 R 4	0—4 in.	A	1·67	4·93	1·17	0·077	15·2	6·4	80·39	4·80	9·19	0·48	0·159
„	4 in.—3 ft.	B	1·20	3·04	0·33	0·034	9·6	6·6	83·68	4·80	7·20	0·39	0·084
„	3—5 ft.	C	1·22	3·11	0·11	0·036	3·1	6·8	83·20	5·04	6·31	0·24	0·007

TABLE III

Analytical results of terraced brown earths—sandy soils

Mechanical

Name of profile	Depth	Horizon	Determinations						
			Stones and gravels (per cent)	Coarse sand (per cent)	Fine sand (per cent)	Silt (per cent)	Clay(per cent)	Exchange H.m.e. (per cent)	Per cent base saturation
13 R 4	0—4 in.	A	15·9	34·00	33·06	14·55	17·65	0·088	98·53
	4 in.—3 ft.	B	34·8	40·17	36·53	7·65	12·00	0·088	98·27
	3—5 ft.	C	37·9	43·30	43·40	7·500	5·40	0·875	72·66

TABLE IV

Analytical results of terraced brown earths—loamy soils

Chemical

Name of profile	Depth	Horizon	Determinations											
			Moisture (per cent)	Loss on ignition (per cent)	Organic carbon (per cent)	Organic nitrogen (per cent)	C/N	pH	Insoluble matter in strong HCl (per cent)	Fe_2O_3 (per cent)	Al_2O_3 (per cent)	R_2O_3 (per cent)	MgO (per cent)	CaO (per cent)
X18 Y7	0—6 in.	A	1·9	3·70	0·70	0·07	10·03	5·8	79·8	5·40	8·20	13·6	0·10	0·049
	6 in.—1 ft. 6 in.	B	2·5	3·44	0·31	0·06	5·16	5·3	77·1	6·20	10·00	16·2	0·07	0·028
	1 ft.6 in.—2 ft. 10 in.	C	1·3	2·23	0·39	0·05	7·80	5·5	82·4	5·68	6·82	12·5	0·15	0·035

Table V

Analytical results of terraced brown earths—loamy soils

(Mechanical analysis of 2 mm. sample)

Name of profile	Depths	Horizon	Determinations						
			Stones and gravels (per cent)	Coarse sand (per cent)	Fine sand (per cent)	Silt (per cent)	Clay (per cent)	Exchange H. m. e. (per cent)	Per cent base saturation
X18 Y 7 . .	0—6 in. . .	A	10·8	12·71	49·67	20·75	16·25	*Nil.*	100
	6 in.—1 ft. 6 in. .	B	5·6	10·56	27·60	29·40	23·10	0·613	94·38
	1 ft. 6 in.—2 ft. 10 in.	C	29·9	46·82	39·84	4·60	8·70	0·700	87·72

Table VI

Analytical results of terraced brown earths—clayey soils

(Chemical)

Name of profile	Depth	Horizon	Determinations											
			Moisture (per cent)	Loss on ignition (per cent)	Organic carbon (per cent)	C/N	pH	Insoluble matter in strong HCl (per cent)	Fe_2O_3 (per cent)	Al_2O_3 (per cent)	MgO (per cent)	CaO (per cent)	R_2O_3 (per cent)	Organic nitrogen (per cent)
15 R 3	0—5 in.	A_1	3·05	5·83	1·79	15·2	6·3	75·80	5·60	7·82	0·33	0·131	13·42	0·118
	5 in.—1 ft. 6 in.	A_3	3·06	4·20	0·62	9·9	5·6	76·74	6·08	7·74	1·32	0·131	13·82	0·063
	1 ft. 6 in.—2 ft. 8 in.	B	3·34	3·64	0·22	5·6	5·3	77·64	6·24	7·51	1·08	0·084	13·75	0·039
	2 ft. 8 in.—3 ft. 6 in.	B+C	3·63	4·11	0·30	6·4	5·1	76·63	5·92	7·71	0·72	0·112	13·63	0·046

Table VII

Analytical results of terraced brown earths—clayey soils

(Mechanical analysis of 2 mm. sample)

Name of profile	Depth	Horizon	Determinations						
			Stones and gravels (per cent)	Coarse sand (per cent)	Fine sand (per cent)	Silt (per cent)	Clay (per cent)	Exchange H. m.e. (per cent)	Per cent base saturation
15 R 3 . .	0—5 in. . .	A_1	3·0	6·76	29·65	33·00	29·80	0·612	95·02
	5 in.—1 ft. 6 in. .	A_3	...	0·20	27·23	43·25	29·75	4·46	60·18
	1 ft. 6 in.—2 ft. 8 in.	B	...	0·39	27·19	41·45	31·45	8·05	48·06
	2 ft. 8 in.—3 ft. 6 in.	B+C	...	0·44	24·17	39·50	35·25	8·05	64·85

It is evident both from visual observations and analytical data given above that these profiles are typical of brown forest soils, notwithstanding the fact that considerable disturbances have been brought about by cultivation and terracing operations.

Transitional podsols

The authors have already given a complete account of visual profile characteristics and relevant analytical data for some of the typical podsol formations at Chaubattia under unterraced natural conditions. A large number of profiles showing podsolic tendencies have now been studied in the terraced portions of the orchard. The visual characters of two such typical profiles are recorded below :—

Profile	Depth	Horizon	Description
X15 Y15	0—8 in.	A_1	Grey ; granular ; loamy ; dark grey when wet.
	8 in.—2 ft. 8 in.	..	Same as above in air-dried condition but slightly darker *in situ*.
	2 ft. 8 in.—3 ft. 5 in.	A_2	Silty loam ; laminated ; structureless ; yellowish ; brownish yellow when wet.
	3 ft. 5 in. and below	B	Hard ; brownish yellow, clayey soil ; deep brown when wet. Whitish incrustations round prismatic soil mass. Hard pan below.
X12 Y21	0—1 ft. 2 in.	A_1	Organic ; silty clay ; when wet brownish grey ; granular.
	1 ft. 2 in.—2 ft. 2 in.	A_2	Organic loam ; brown ; coarsely granular ; when wet reddish brown.
	2 ft. 2 in.—3 ft. 1 in.	A_3	Organic clayey with mica rock pieces ; platy yellowish brown ; when wet dark brown.
	3 ft. 1 in.—3 ft. 7 in.	B	Clayey ; whitish incrusations ; brown ; when wet deep brown ; hard ; prismatic ; micaceous.
	3 ft. 7 in.—4 ft. 8 in.	B+C	Yellowish brown ; when wet brown ; micaceous sandy ; indurated with infiltered clay.

The whitish cementations in the B horizon of both these profiles round prismatic soil mass and the platy or laminated nature of a part of the A horizon indicate as far as visual observations go the podsolic nature of both the profiles. Chemical and mechanical analyses of both the profiles are given in Tables VIII and IX.

TABLE VIII

Analytical results of terraced podsolic soils

(Chemical determinations)

Name of profile	Depth	Horizon	Moisture (per cent)	Loss on ignition (per cent)	Organic carbon (per cent)	Organic nitrogen (per cent)	C/N	*p*H	Insoluble matter in strong HCl (per cent)	Fe_2O_3 (per cent)	Al_2O_3 (per cent)	R_2O_3 (per cent)	$\frac{SiO_2}{R_2O_3}$	MgO (per cent)	CaO (per cent)
X15 Y15	0—8 in.		3·45	7·19	2·22	0·21	10·56	6·3	71·01	5·09	10·45	15·54	4·57	1·25	0·358
	8 in.—2 ft. 8 in.	A_1	4·18	8·49	3·00	0·19	15·77	6·1	69·14	5·23	10·97	16·20	4·26	1·44	0·329
	2 ft. 8 in.—3 ft. 5 in.	A_2	2·65	2·97	0·27	0·06	4·31	5·6	77·58	4·61	9·86	14·47	5·36	1·07	0·105
	3 ft. 5 in. and below.	B+C	3·02	2·56	0·34	0·06	5·21	5·6	76·95	3·86	10·13	13·99	5·50	1·53	0·238
X12 Y21	0—1 ft. 2 in.	A_0	2·94	7·13	2·11	0·18	11·7	6·7	73·74	5·09	9·29	14·38	5·13	0·90	0·308
	1 ft. 2 in.—2 ft. 2 in.	A_1	3·05	3·53	1·74	0·07	24·9	6·2	76·76	5·83	8·16	13·99	5·49	1·52	0·175
	2 ft. 2 in.—3 ft. 1 in.	A_2	2·68	3·50	0·67	0·05	13·4	5·5	77·70	4·95	9·53	14·48	5·37	1·18	0·098
	3 ft. 1 in.—3 ft. 7 in.	B	3·13	3·00	0·65	0·05	13·0	5·4	77·70	4·99	9·02	14·01	5·90	1·07	0·063
	3 ft. 7 in.—4 ft. 8 in.	B+C	1·12	2·73	0·77	0·03	25·7	6·0	82·87	4·85	7·04	11·89	6·97	0·58	0·035

TABLE IX

Analytical results of terraced podsolic soils

(Mechanical determinations)

Name of profile	Depth	Stones and gravels (per cent)	Coarse sand (per cent)	Fine sand (per cent)	Silt (per cent)	Clay (per cent)	Exchange H. m. e. (per cent)
X15 Y15	0—8 in.	0·3	1·60	28·31	47·20	26·50	0·26
	8 in.—2 ft. 8 in.	0·2	0·53	20·82	42·65	28·95	0·96
	2 ft. 8 in.—3 ft. 5 in.	...	0·95	24·97	43·10	24·10	5·78
	3 ft. 5 in. and below	...	0·32	23·02	44·35	26·15	3·32
X12 Y21	0—1 ft. 2 in.	4·4	6·78	33·03	32·05	24·20	0·044
	1 ft. 2 in.—2 ft. 2 in.	...	1·24	32·54	38·20	25·90	0·131
	2 ft. 2 in.—3 ft. 1 in.	8·1	4·32	35·82	37·10	21·60	0·175
	3 ft. 1 in.—3 ft. 7 in.	10·7	4·85	32·53	36·80	24·85	1·312
	3 ft. 7 in.—4 ft. 8 in.	5·0	16·17	45·73	15·30	20·15	1·750

It is evident from Tables VIII and IX that in spite of morphological characteristics pointing to the similarity of these soils with transitional podsols there is hardly any general indication of colloidal matter, clay, organic carbon and lime having been eluviated from A horizon. Similarly iron and alumina do not tend to have undergone any eluviation whatsoever. The trend of changes in regard to *p*H and exchange acidity also shows that these profiles have fundamental characteristics of brown forest soils. With a view to be able to elucidate further the nature of these profile formations, it was considered necessary to analyse the clay fractions of these soils. The results of clay fraction analysis are given in Table X.

TABLE X

Analysis of clay fraction—terraced podsolic soils

Profile	Depth	Horizon	SiO_2 (per cent)	Fe_2O_3 (per cent)	Al_2O_3 (per cent)	$\frac{SiO_2}{Al_2O_3}$	$\frac{SiO_2}{R_2O_3}$	$\frac{Al_2O_3}{Fe_2O_3}$
X15 Y15	0—8 in.	...	47·20	14·37	28·63	2·734	2·116	3·119
	8 in.—2 ft. 8 in.	A_1	47·62	14·37	26·03	3·101	2·292	2·836
	2 ft. 8 in.—3 ft. 5 in.	A_2	50·88	13·17	22·43	3·845	2·796	2·666
	3 ft. 5 in. and below.	B+C	51·72	13·17	21·43	4·091	2·937	2·547
X12 Y21	0—1 ft. 2 in.	A_0	45·90	15·17	19·63	3·962	2·653	2·026
	1 ft. 2 in.—2 ft. 2 in.	A_1	47·78	13·57	21·73	3·726	2·664	2·507
	2 ft. 2 in.—3 ft. 1 in.	A_2	49·58	13·97	19·33	4·346	2·974	2·166
	3 ft. 1 in.—3 ft. 7 in.	B	52·50	12·78	19·32	4·606	3·237	2·367
	3 ft. 7 in.—4 ft. 8 in.	B+C	48·32	15·97	19·13	4·281	2·792	1·875

Although there is an indication of accumulation of Fe_2O_3 in the lower horizons of profile X12 Y21 it is clear from the table above that there is no general

tendency of eluviation of the sesquioxides in the profiles. On the other hand the constancy of $\frac{SiO_2}{R_2O_3}$, $\frac{SiO_2}{Al_2O_3}$ and $\frac{Al_2O_3}{Fe_2O_3}$ ratios throughout the solum shows that profiles such as these are typical brown forest soils [Robinson, 1930; Mukerji and Das, 1940]. The high ratio of $\frac{SiO_2}{R_2O_3}$ establishes clearly the fact that weathering in these profiles is primary. Thus it is quite evident that profile X15 Y15 is a typical brown forest soil and profile X12 Y21 has podsolic tendency.

The reasons for these podsolic profiles showing some brown forest soil characters will be clear from a consideration of the percentage base saturation figures of the profiles given in Table XI. It will be noticed that in spite of high base saturation of the surface layers the lower layers are still showing considerable degree of unsaturation, specially in the case of profile X15 Y15. This indicates that the process of transformation of the podsols into brown forest soils due to terracing is in some cases not quite complete.

On a joint consideration of the data given in Tables X and XI, and particularly because of the presence of silicious material round structural soil mass of B horizons, these soils have been classified as transitional podsols, where it appears that the dynamics of the podsolic soil formations have undergone a reversal to brown forest soil type under terraced conditions. Although on the average the base exchange capacity of these soils is rather low, the high percentages of base saturation of the surface soils may be responsible for bringing about a reversal of these podsolic soils.

TABLE XI

Percent base saturation—terraced podsolic soils

Profile	Depth	Horizon	*p*H	Exchange acidity (m. e. per cent)	Base-exchange capacity (m. e. per cent)	Base-saturation (per cent)
X15 Y15	0—8 in.	...	4·2	0·26	15·2	98·29
	8 in.—2 ft. 8 in.	A_1	4·0	0·96	16·9	94·32
	2 ft. 8 in.—3 ft. 5 in.	A_2	3·6	5·78	13·7	57·81
	3 ft. 5 in. and below.	B+C	3·6	3·32	22·2	85·05
X12 Y21	0—1 ft. 2 in.	A_2	4·7	0·044	14·3	99·69
	1 ft. 2 in.—2 ft. 2 in	A_1	4·1	0·131	12·5	98·95
	2 ft. 2 in.—3 ft. 1 in.	A_2	3·9	0·175	11·6	98·49
	3 ft. 1 in.—3 ft. 7 in.	B	3·8	1·312	21·6	93·93
	3 ft. 7 in.—4 ft. 8 in.	B+C	3·9	1·75	8·7	79·89

GENERAL DISCUSSION

In the foregoing pages the results of a critical study of soil types found under terraced and cultivated conditions at Chaubattia have been given. In spite of the disturbances caused to the soil profiles by terracing and cultivation it is interesting to note that the essential features of brown forest soils have persisted. Podsolic formations, on the other hand, seem to have

undergone changes, and show certain characteristic features of brown forest soils. A highly base-sturated A-horizon is one of the peculiar characteristics of these profiles. This high base-saturation can be attributed above all to the following two reasons :—

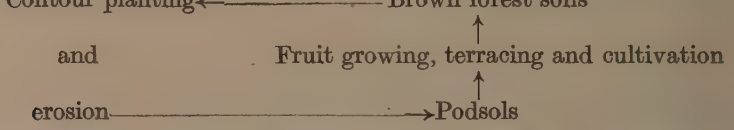
(*i*) Due mainly to the manuring practices, considerable amount of organic matter and bases are added to the surface soils ; and

(*ii*) leaf fall of fruit trees adds considerable amount of bases and humus to the surface soil, and these at the time of annual digging of the terraces are thoroughly incorporated into the soil.

From the data presented in this paper it is clear that brown forest soil is the only soil type which is in equilibrium with the diverse factors underlying the raising of temperate fruits by terracing and cultivation. Had it not been so some of the characteristics in the dynamics of brown forest soil formation should have been modified to suit the altered conditions.

Podsolic formations being unstable under the conditions peculiar to the growing of fruit trees in these hills, have assumed brown forest soil features which are much more suitable for fruit growth under the conditions generally prevailing in the Kumaun hills.

The pedogenic processes giving rise to the formation of brown forest soils are somewhat similar to those causing the development of podsols, and the latter appear to be the end product of a series of natural operations that bring about the formation of brown forest soils at an intermediate stage. The brown forest soil characteristics of the terraced podsolic soils studied by us can only be ascribed to a reversal of this process taking place under terraced and cultivated conditions. This hypothesis clearly explains why under terraced and cultivated conditions a fully developed podsolic formation is rarely met with. The dynamics of the soil formation under this multiphase system of fruit growing can be represented graphically as follows :—



Terracing as an agronomic practice is followed all over the world for the profitable utilization of soils which are liable to severe erosion. Different types of terraces have, therefore, been found suitable under different topographical and climatic conditions. Terracing and cultivation on terraces along the hill slopes as practised in Kumaun have their peculiar problems, particularly where these hill soils are utilized for the development of fruit cultivation.

The detailed examination of 716 profiles under different gradients shows that topographically immature sandy and stony soils are found mostly along slope gradients of 45° or more. The subsoils of all such immature profiles remain light, loose, and unconsolidated under terraced conditions with the result that the finer soil material of the subsoil gets washed away during monsoon and the water retaintivity of the profile as a whole becomes on that account very low. Due to this and other incidental factors the growth of apple and other fruit trees in such localities of immature soil formation is very

poor, and in dry periods signs of leaf drooping and set back in growth are very common. It will thus appear that utilization of such topographically immature soils is not likely to yield the desired effect.

Summary

1. Terraced soils studied at the Government Orchard, Chaubattia in the Kumaun hills show that the textural characters of profiles depend on the slope gradients. Sandy soils are found mostly along gradients of 45° or more ; whereas, loamy and clayey soils are usually met with under milder slope conditions.

2. The genetic soil type met with under terraced conditions are mostly brown forest soils, textural characters of the profiles of which depend on slope gradients.

3. Terracing and cultivation as practised for fruit growing tend to make podsolic soil types assume certain characteristics of brown forest soils.

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FRACTIONATION OF PHOSPHORIC ACID IN ORGANIC MANURES*

BY

M. O. GHANI, M.Sc., PH.D.(LOND.)

Department of Soil Science, Dacca University

(Received for publication on 11 March 1941)

IT has been found by various workers that a considerable portion of the total phosphorus of grassland and fen soils is in organic form. The organic phosphorus of arable soils is comparatively small and is mainly derived from the crop residues and from organic manures used as fertilizers. The fate of the organic phosphorus upon entering the soil and the form into which it is ultimately converted are yet uncertain and controversial [Ghani, 1938].

It is, therefore, interesting to know the proportion of organic and inorganic phosphorus in such manures and the form in which they are present in them. Such an analysis may give useful information about the fertilizing value of the organic manures.

Funatsu [1908] determined the phosphoric acid in the form of lecithin and nuclein and in a form soluble in dilute hydrochloric acid in several manure cakes and found that the amounts of lecithin and nuclein were comparatively small. Tsuda [1909] made a quantitative determination of different forms of phosphoric acid in several organic manures of vegetable and animal origin. He found that the animal manures had their phosphorus mainly in the inorganic form and the vegetable ones mainly in the organic form.

EXPERIMENTAL

Samples of poultry manure (both fresh and kiln dried), farmyard manure and Adco compost were analysed by a method of fractionation similar to that of Tsuda. Determination of P_2O_5 in the various fractions was made by the colorimetric method of Deniges [1920] as modified by Truog and Meyer [1929]. The outline of the method adopted is given below schemetically.

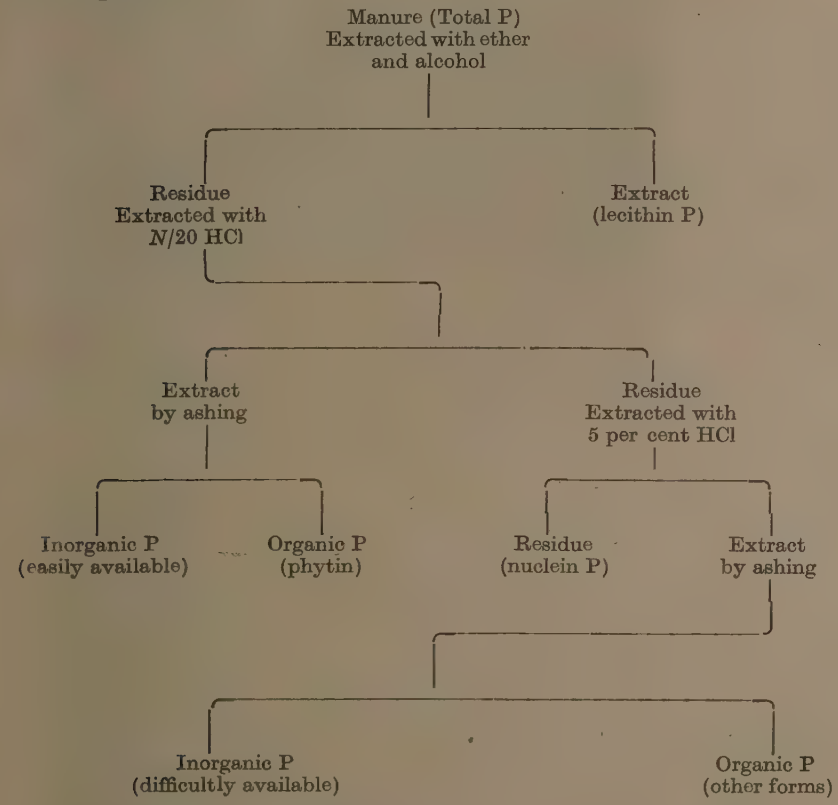
The procedure of analysis was as follows :—

5 gm. of the material were extracted for 20 hours with ether in a soxhlet apparatus. The residue was similarly extracted with absolute alcohol for a period of 12 hours. The two extracts were mixed, evaporated to dryness and the P_2O_5 in the residue determined colorimetrically after gentle ignition. This represents phosphoric acid in the form of lecithin.

The residual material from the alcoholic extraction was dried and extracted with 200 c.c. of $N/20$ hydrochloric acid by shaking for two hours. The inorganic P_2O_5 was determined in an aliquot of the extract. The total P_2O_5 in the extract was determined by evaporating an aliquot with 2 c.c. of

*The work reported here was carried out at the Rothamsted Experimental Station, England.

10 per cent solution of magnesium nitrate and by igniting it gently. The ignited residue was treated with 1 c.c. of conc. hydrochloric acid, diluted with water to about 20 c.c., heated on a sand-bath for 15 minutes, made up to a known volume and P_2O_5 determined in an aliquot. The difference between total and inorganic P_2O_5 gave the organic phosphorus in the extract. The organic portion represents phytin and the inorganic portion represents easily available phosphorus.



The residue of the $N/20$ hydrochloric acid extraction was dried and again extracted with 5 per cent hydrochloric acid in the same way and its P_2O_5 both organic and inorganic, were determined as before. The inorganic phosphorus represents phosphorus in difficultly available form and the organic fraction represents combination other than lecithin, phytin and nuclein.

The last residue was dried and total P_2O_5 determined by ashing. This represents phosphoric acid in the form of nuclein.

The total phosphorus in the manure was obtained by summing all the fractions. An independent determination in the manure was found to agree, within experimental error, with the summation figure. The results are shown in Table I.

TABLE I

Fractions of P_2O_5 in organic manures expressed as percentage total P_2O_5

	Poultry manure* (dried)	Poultry manure (fresh)	Farmyard manure	Adco compost
P_2O_5 soluble in ether and alcohol	0·8	0·3	0·4	0·1
P_2O_5 soluble in $N/20$ hydrochloric acid — Inorganic (easily available)	40·5	43·3	68·5	26·3
P_2O_5 soluble in $N/20$ hydrochloric acid — Organic phytin)	35·1	32·8	4·6	0·0
P_2O_5 soluble in 5 per cent hydrochloric acid — Inorganic (difficultly available)	4·2	4·5	7·2	66·3
P_2O_5 soluble in 5 per cent hydrochloric acid — Organic (other forms)	12·7	12·1	5·0	1·1
P_2O_5 in the residue (nuclein)	6·7	7·0	14·3	6·2
Total organic P_2O_5	55·3	52·2	24·3	7·4
Total inorganic P_2O_5	44·7	47·8	75·7	92·6

*Commercially prepared sample of dried manure

It will be seen from the table that the greater part of the phosphorus of poultry manure is in organic form, that 25 per cent of the phosphorus of farmyard manure is organic, whereas Adco compost has its phosphorus mostly in the inorganic form. Most of the inorganic phosphorus of the poultry manure and the farmyard manure was dissolved in the dilute acid (that is easily available to plants), whereas in the case of Adco compost about 70 per cent of the inorganic phosphorus was insoluble in dilute acid but was soluble in 5 per cent hydrochloric acid. This would mean that Adco compost is inferior to the natural manures so far as the availability of its phosphorus is concerned. Of the organic phosphorus compounds, phytin is the highest fraction in the poultry manure, whereas farmyard manure and Adco compost have their organic phosphorus mainly in the nuclein fraction. The high content of phytin phosphorus in poultry manure and its absence in Adco compost are readily understandable from the consideration that phytin is present in seeds and grains in large amounts (70-90 per cent of the total phosphorus is in this form) which constitute the major portion of the feed of these birds. Lecithin is present in small amounts in all these manures. It is also evident from the results that fresh and commercially prepared samples of poultry manure behave vary similarly and that kiln drying has not produced any appreciable change in the different fractions.

The very interesting fact that emerges out of this analysis is that about 70 per cent of the total phosphorus of farmyard manure is easily available to plants. The classical experimental plots at Rothamsted and Woburn offer an excellent field for testing this point further. For this purpose samples from the ' no-manure ', ' mineral ' and the ' dung ' plots were analysed for their content of available phosphorus and organic phosphorus. Available phosphorus was determined in a semi-normal acetic acid extract of the soil and the organic phosphorus in a quarter normal sodium hydroxide extract by the bromine oxidation method of Dean [1938] as modified by Ghani [1938].

The mineral manured Broadbalk wheat plots at Rothamsted received 3·5 cwt of superphosphate per acre annually since 1843, while that at Woburn (continuous Barley) received 3·5 cwt superphosphate per acre annually for the first 30 years and 3·0 cwt superphosphate per acre from 1907 to 1926. The dunged plots at Broadbalk received annually 14 tons of farmyard manure per acre containing an amount of phosphorus roughly equivalent to 3·5 cwt of superphosphate and that at Woburn the annual dressing was roughly equal to 2·5 cwt of superphosphate per acre. The results of analysis are shown in Table II.

TABLE II

Effect of the farmyard manure on the available phosphorus and the organic phosphorus of the soil

(Mg. P_2O_5 per 100 gm. soil)

Soil	Treatment	Acetic acid-soluble P_2O_5	Organic P_2O_5	*p*H
Rothamsted				
A 3957	No manure	Trace .	21	7·9
A 4279	Minerals .	40	20	7·8
A 3956	Dung .	54	30	7·5
Woburn				
A 3009	No manure	3	36	5·4
A 3003	Minerals .	16	32	5·8
A 3019	Dung .	27	48	5·8

It will be seen from the table that the acetic acid-soluble phosphorus in two soils which receive no manure is very small. The mineral manured plots, both at Rothamsted and Woburn give higher values for this fraction, while the two dunged plots give still higher values and in fact are outstandingly rich in the acetic acid-soluble fraction. This high availability figure is quite in keeping with the results obtained in the previous fractionation of the manure.

Considering the extra amount of farmyard manure, the Broadbalk plot received in the first 33 years (before experiment at Woburn was started) and the lesser amount of annual dressing in the last 20 years, it would appear that the accumulation of organic phosphorus due to the application of dung proceeds at a higher rate in the Woburn plot than in the Broadbalk plot. This suggests that organic phosphorus compounds are more stable under acid conditions due to consequent lack in microbial activities. This, together with the fact that acetic acid-soluble phosphorus in the Rothamsted plot is double that of the Woburn plot, further suggests that organic phosphorus of farmyard manure is more quickly mineralized in neutral soils than in acid ones.

Summary

1. A quantitative study of the distribution of different forms of phosphoric acid in poultry manure, farmyard manure and Adco compost has been made.

2. The greater part of the phosphorus of poultry manure is in organic form ; phytin constitutes a major portion of its organic phosphorus and its inorganic phosphorus is present mostly in the available form. 75 per cent of the phosphorus of farmyard manure is inorganic, most of which is easily available. The Adco compost has its phosphorus mainly in the inorganic form of which the greater part is difficultly available. Lecithin is small in all the three manures, while nuclein is comparatively high in the farmyard manure.

3. Analyses of soils from the experimental plots at Rothamsted and Woburn show that application of farmyard manure maintains the available phosphorus status of a soil at a high level and that the organic phosphorus of farmyard manure is fairly quickly mineralized in neutral soils.

Acknowledgments

The author wishes to acknowledge his thanks to the Director and staff of the Rothamsted Experimental Station for supplying the manure samples and for providing facilities for this work.

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DAMAGED LANDS IN THE DECCAN AND THEIR CLASSIFICATION*

BY

R. P. TALATI, M. Ag.

Poona Irrigation and Research Division

(Received for publication on 16 December 1940)

(With Plates LXVII and LXVIII and seven text-figures)

THE Deccan tract can be classified into three main groups as under :—

(1) The Ghats
(2) The transition tract
(3) The Desh tract

The Ghats receive very heavy rainfall varying from 100 to 150 in. The transition tract receives from 20 to 30 in. of rain while the Desh on the eastern side receives rainfall varying normally from about 20 to 25 in. and is often frequented with famine. In order to relieve cultivators of their distress, Government constructed dams at suitable sites on the Ghats to serve as storage reservoirs. These dams are the Bhatgar dam feeding the Nira Left and Right Bank Canals to a length of about 100 miles. The Khadakvasla dam feeding the Mutha Canals, and the Bhandardara, Darna and Chankapur dams feeding the Pravara, Godavari and Girna Canals, respectively. Thus, the system spreads through the Poona, Sholapur, Ahmednagar and Nasik districts. Irrigation has done immense good to cultivators as they are now able to grow crops with minimum water charges without any risk. On the other hand, all concerned realize the increasing danger in the spreading of waterlogged and salt-affected lands. We can ascribe this formation due to the following causes :—

(*a*) Weathering and leaching of the parent rock
(*b*) Presence of salts in the soil profile
(*c*) Quality of irrigation water

(*a*) On account of the rapid changes in temperature, some of the minerals of the trap rock weather rapidly and the resulting ingredients are leached away to the low-lying areas. This is the chief reason for the formation of salt lands, [Mann and Tamhane, 1910].

(*b*) The soil profile contains salts in the accumulation horizon which, under conditions of high subsoil water-table resulting from heavy perennial irrigation, rise to the surface and deposit salts.

(*c*) The quality of irrigation water of all Deccan canals is excellent as the salts vary from 10 to 20 parts and rarely 40 parts per 100,000 parts and the *p*H values range from 7·0 to 7·50.

These results show that there is no possibility of formation of barren lands by using these waters alone for irrigation for any number of years.

*Paper read on 7 October 1940 at the Symposium on 'Alkali Soils of the Deccan' under the auspices of the Indian Society of Soil Science with suitable modifications.

Causes of rise of subsoil water-table

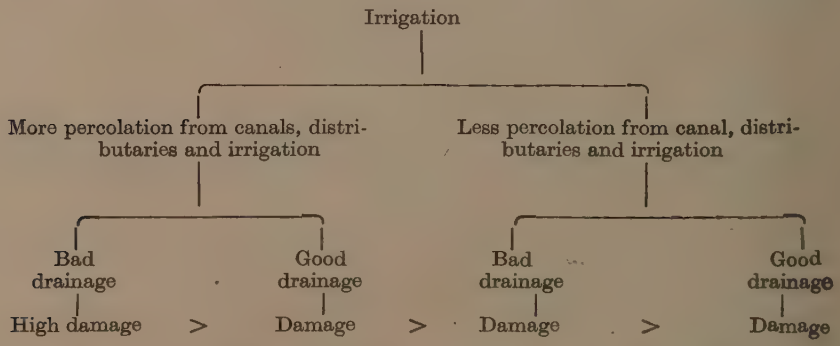
The canals generally pass through murrum cuttings in the ridges and in embankment in the valleys (to straighten as far as possible zigzag course of a falling contour) and as a result there is percolation through the banks. There is also percolation from the irrigated fields particularly through the shallower soils and this is incurred due to over-irrigation.

The percolation water from these sources passes into the subsoil and adds to the residual subsoil water raising its level. The subsoil water then moves through the pervious subsoils towards the valleys to find an outfall in the *nalla*. The subsoil water level consequently rises to form a gradient—sufficient to pass the discharge and where it reaches the surface, it causes water-logging, i.e. free water at the surface. Where owing to the relative impermeability of the intervening subsoils the rate of capillary rise of water through the soil is less than the rate of evaporation from the surface the salts from solution deposit on the surface and concentrate in the upper layers.

It will, therefore, be seen that damage results from surplus subsoil water which causes a rise in the subsoil water-table and evaporates from the surface. Hence, damage varies directly with the difference in quantity of water (discharge) passing into the subsoil and that coming out of the subsoil into the *nallas*.

Again, water passing into the subsoil is equal to the percolation from irrigation plus percolation from canals and distributaries. Water coming out of the subsoil, through *nallas* is proportional to the draining quality of the valley. It follows that there will be more damage with more irrigation, the drainage being constant, and less damage with better drainage, irrigation being constant.

This can be diagramatically represented as under :—



There will be more percolation in catchments where there are larger areas under irrigation under comparatively shallower soils and less percolation with the same amount of irrigation in catchments having mostly deeper soils.

Similarly, percolation will be more from canal and distributary in murrum section than the soil section as murrum is more pervious to water than soil.

Damage under Deccan canals

Table I will give an idea of the extent of damage on different canals. It will be seen that there is a considerable area under damage on each canal and in addition 1½ times the area under 4 ft. hydroisobath (equal depths of water from ground level).

Table I

Name of canal	Damaged area in 1928-30 in acres	Damaged area in 1938-40 in acres
Nira Left Bank	9,407	17,942
Nira Right Bank	909	9,653
Godavari canals	17,000	25,047
Pravara canals	13,407	22,442

Scope of reclamation

These lands which are now spoiled were once very valuable, producing good crops. Their value can be put at Rs. 200 to Rs. 400 per acre in normal years, the price varying according to the proximity or otherwise of towns. This shows how important it is to tackle the problem and try to bring back these lands once again under cultivation. The first attempt, therefore, consists of providing drainage by laying pipes or providing open drains.

Subsoil drainage

As already stated, in the Deccan the canal starting from a pick-up weir follows a falling contour crossing several subsidiary ridges and valleys in its course.

The soils and the subsoils on the ridge slopes are usually open and pervious whereas those in the valley are more or less heavy and impervious. Hence, unless the pervious subsoil is continuous up to the natural *nalla*, drainage of the valley is rather difficult. If the pervious layer dips down below the existing *nalla* due to the silting of the old valley, as is common in the Deccan, drainage is blocked and an outfall to the subsoil water, entrapped in the pervious layer, has to be provided by artificial drains, connecting the pervious subsoil with the natural outfall at the lowest level possible. On this account, a drain passes partly through a pervious strata and partly through an impervious one. It is the former portion that actually drains the land and reduces water pressure acting in the area lower down ; whereas the drain in the latter portion merely carries water. Pipes are usually laid in the pervious portion whereas the drain in the carrier portion is kept open. For this purpose a very careful soil, subsoil and hydro-logical survey is carried out by means of levelling instruments, auger bores and dionic water tester. The scheme of drainage is carefully planned from this data.

Fig. 1 shows the scheme of drainage in the Manjri area, a village about 10 miles from Poona. It shows murrum isobath, damaged area and the natural *nalla*.

FIG. 1. Plan showing subsoil survey and damaged area classification and natural *nalla*, Mutha Right Bank Canal, Manjri Drainage Scheme (scale 1 in. = $\frac{5}{8}$ mile)
(Area enclosed in hatched line is all damaged)

Fig. 2 shows the same scheme as completed whereby the remnant subsoil water-table existed only as a very small fraction of what it was originally.

Plate LXVII, fig. 1 shows the operation of laying pipes in a drain, 5 ft. below ground level.

FIG. 1. Operation of laying pipes in a drain (Mark the top string in level with the ground)

FIG. 2. Pipe-line after completion and discharging in open drain

Sugar cane crop. Local cane (Pundia). Tried in a mixed saline soil in S. No. 128, Baramati

(Promising crop 8-10 feet tall)

FIG. 2. Plan showing 4 ft. H. I. Bs. before and after drainage and drainage lines, open and closed (pipe lines), Mutha Right Bank Canal, Manjri Drainage Scheme (Scale 1 in. = $\frac{1}{8}$ mile)

Plate LXVII, fig. 2 shows the same pipe line after completion and discharging in the open drain.

Detailed scientific work on drainage in the Deccan is described by Inglis and Gokhale [1927] and its engineering technique by Evershed [1937].

Reclamation

The next step is reclamation of the areas where the subsoil water-table is lowered as a result of drainage.

Soil scientists like Hilgard, Gedroiz, De Sigmond, Burgess and Breezeale and others have tried various fertilizers and recommended use of gypsum. Marr [1927] describes also the role of CO_2 in alkali reclamation. In India,

the science of reclamation is gradually developing. Taylor and Puri [1935] of the Punjab Irrigation Research Institute suggest the following :—

(*a*) By a suitable crop rotation in which rice is used as an agent for decomposing the sodium clay

(*b*) The application of gypsum which introduces sufficient calcium ions in solution to prevent the base-exchange reaction between sodium salt and a calcium clay

Dr Dhar has proposed a new method of reclamation of alkali soil by applying molasses varying from 100 to 500 maunds to as high as 1,000 maunds per acre. This, when added to alkali soils and watered, converts alkali soils into acidic ones. At present it has scope only near the sugar factories. Besides, the cost is prohibitive as in the Deccan the rates quoted are eight annas per maund.

Coming to the Deccan, Mann and Tamhane [1910] have described salt lands in the Nira valley. Their work deals with the salts found in the valley in a very general way. Since this publication, our knowledge on salt lands is much advanced which is put up in this paper.

Damaged lands and their classification

It is mentioned that there is an extensive area under damage amounting to about 75,000 acres with almost an equal area where water-table is within 4 ft. from ground level. Out of this, one third area is damaged due to water-logging and the remaining area is damaged due to salt as a result of capillary action from high saline subsoil water-table.

The soil types beginning from coarse soils and medium black soils are not damaged as the deep black soil for the simple reason that they are in the upper reaches or on the ridges. If we measure maximum slope of the different soils at right angles to the contour, we find in normal catchments that the coarse soils have slopes below 1 in 60, the murrum black soils have slopes below 1 in 100 and the deep soils have slopes below 1 in 300 with a fall of say 35 ft. or more from coarse soil to deep soil.

Fig. 3 gives an idea of the usual slope of these soil types. This is a very dominant factor in causing damage.

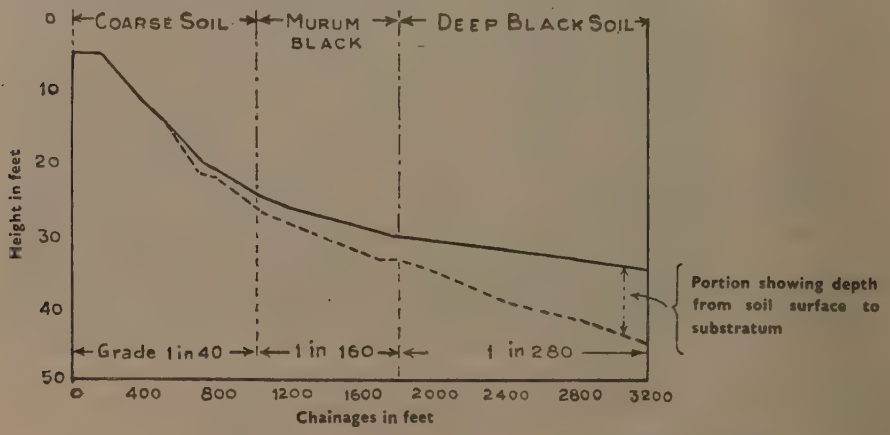


Fig. 3. Normal slopes of different soil types in the Bombay-Deccan

In order to see the actual field conditions of salt distribution several soil profiles were examined from deep soil areas. Analysis of a typical one is given in Table II.

Table II

Results of saline profile from deep black soil type

Serial No.	Ingredients	Depth of soil					
		0—6 inches	6—12 inches	1—2 feet	2—3 feet	3—4 feet	4—5 feet
1	Total soluble salts . . .	3·11	2·28	1·82	1·62	1·14	0·85
	Percentage on total salts						
2	$CaCO_3$	0·93	2·58	4·61	5·60	5·98	6·84
3	$CaSO_4$	29·04	24·59	12·82	8·08	5·25	6·51
4	$MgSO_4$	14·70	13·43	17·31	15·37	19·31	11·79
5	Na_2SO_4	39·57	44·85	48·49	51·44	49·45	53·66
6	NaCl	12·39	16·50	12·18	15·90	16·34	13·75
7	pH values (colorimetric) . .	7·50	7·70	8·00	8·30	8·50	8·65

The results show that the soluble salts consist of mixed salts of calcium, magnesium and sodium. The percentage of sodium salts on the whole shows a tendency to increase with depth.

Similarly several other damaged profiles were examined. Analysis of typical one is given in Table III.

Table III

Results of saline soil profiles from deep grey soil type

Serial No.	Ingredients	Depth of soil						
		0—6 inches	6—12 inches	1—2 feet	2—3 feet	3—4 feet	4—5 feet	7—8 feet
1	Total soluble salts .	2·49	1·23	1·30	1·40	1·18	0·86	0·68
	Percentage on total salts							
2	$CaCO_3$ . . .	1·63	4·43	5·53	5·08	5·35	5·48	6·19
3	$CaSO_4$ . . .	6·03	0·15	2·0	0·96	...	...	...
4	$MgSO_4$ . . .	2·71	6·12	9·0	5·57	10·08	13·87	4·95
5	$MgCO_3$ . . .	1·49	2·33	1·27	3·22	4·15	4·63	4·37
6	Na_2SO_4 . . .	60·37	57·79	49·74	49·55	48·41	45·57	51·47
7	NaCl	24·56	27·13	28·48	30·46	28·65	29·79	32·00
8	pH values (colorimetric)	8·60	8·58	8·72	8·73	8·77	8·93	9·15

It will be seen that the total salts consist mostly of sodium salts. The calcium and magnesium salts are practically negligible. The pH values are on the higher range than those observed in case of the previous profile. Fig. 4 shows the total soluble salts and percentage of different ingredients over total salts in both the types of soils.

Soil profiles containing mixed salts of calcium and sodium were collected and leached of excessive salts. The residual soil was tested for exchangeable bases and other tests with the results given in Table IV

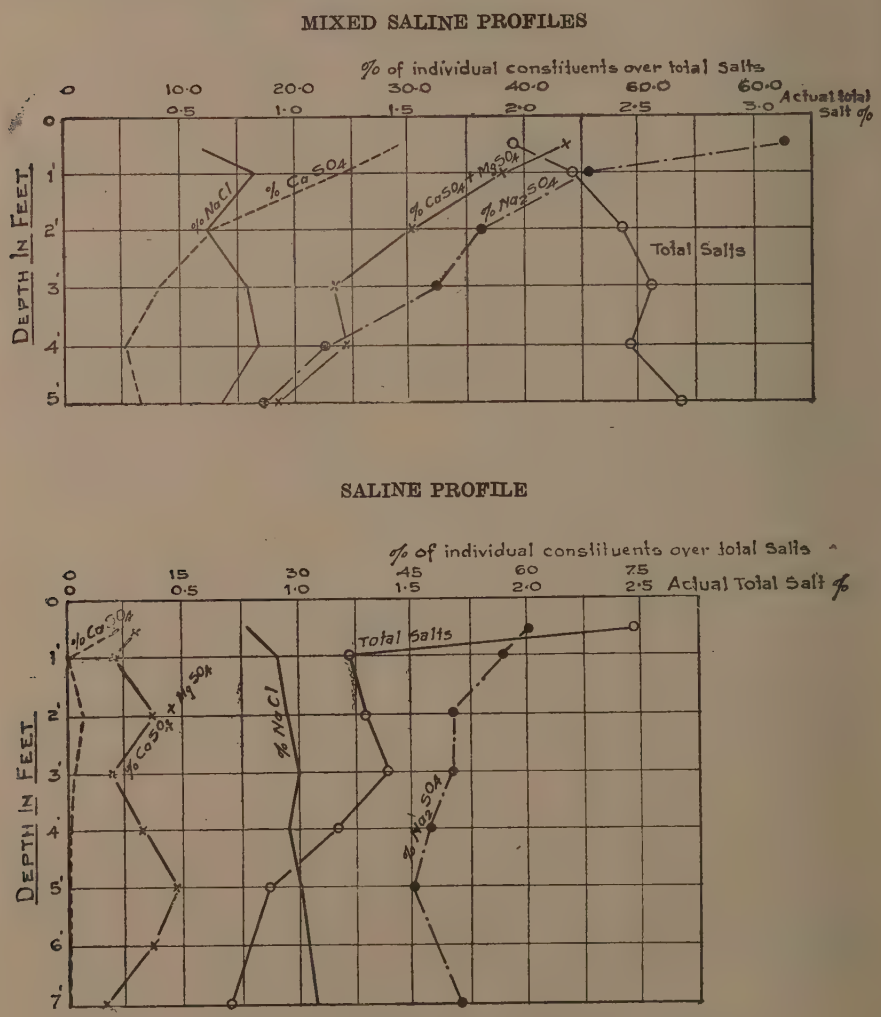


FIG. 4. Percentage of individual constituents over total salts

TABLE IV

Results of soil tests of mixed saline type

Depth	Total* bases milli-equivalents per cent	Per cent monova-lent bases to total bases	Capillary rise (cm.) 300 minutes		*p*H values† in		Original total soluble salts (per cent)
			In water	In NaCl	Distilled water	N-KCl	
I							
0—6 in.	24·92	3·74	3·0	5·9	8·26	7·89	3·64
6—12 in.	24·57	4·89	3·1	6·3	8·28	7·80	1·59
1—2 ft.	24·40	8·02	4·3	6·3	8·46	7·44	0·95
2—3 ft.	24·99	4·04	3·3	4·5	7·94	7·42	1·25
3—4 ft.	23·53	11·57	3·6	4·9	8·02	7·28	0·19
5—6 ft.	26·82	14·01	4·1	5·3	8·04	7·18	0·75
II							
0—6 in.	21·94	1·54	7·8	8·0	7·40	6·96	3·40
6—12 in.	21·56	2·54	3·4	7·2	8·00	7·36	1·04
1—2 ft.	19·05	1·12	2·6	6·3	8·30	7·40	2·64
2—3 ft.	17·91	1·20	2·2	4·7	8·60	7·42	2·28
3—4 ft.	19·18	11·10	1·9	3·7	8·30	7·56	2·10
4—5 ft.	19·66	9·97	2·0	3·4	8·40	7·57	2·64
6—6·5 ft.	...	...	No rise	5·7	9·24	7·62	0·56
III							
0—1 ft.	24·34	11·62	3·0	13·1	8·46	7·57	3·24
1—2 ft.	22·38	14·88	8·4	12·1	8·34	7·64	2·24
2—3 ft.	19·38	10·12	3·2	5·2	8·66	7·56	2·07
3—4 ft.	20·06	15·89	2·7	6·5	8·76	7·57	1·61
4—5 ft.	20·69	31·68	2·6	8·0	9·54	7·64	0·82

*Exchangeable bases were estimated by Puri's method [1935, 1, 2]

†*p*H values were found out by antimony electrode standardized by Puri [1932]

The results of replaceable bases show that the total bases consist of over 95 per cent of divalent bases for top foot of soil, with very low percentage of monovalent bases. The capillary rise shows a fairly good rise throughout. The *p*H values are also low like normal soil profiles of the same type. Fig. 5 clearly illustrates these results.

Similarly, a number of alkali soil profiles where subsoil water level was reduced, were examined. Results of only typical ones are reproduced below :—

ALKALI PROFILE NO. A

This was collected from fine black soil just below murrum black soil. The analysis of water-soluble salts will be clear from Table V.

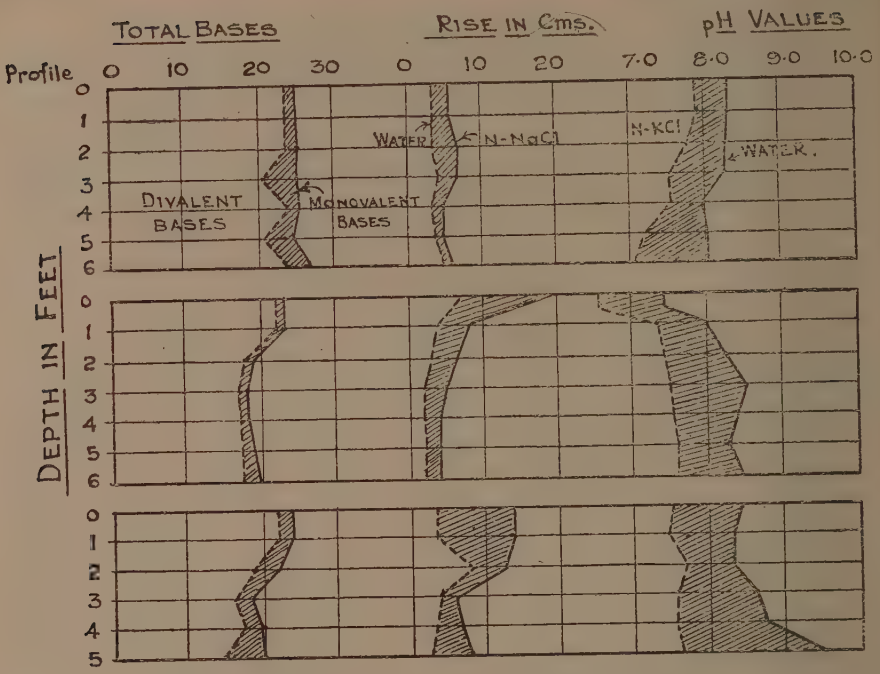


FIG. 5. Showing soil tests in mixed saline soil profile

TABLE V

Results of water-soluble salts of sodiumized fine black soil

Serial No.	Depth of soil	Total soluble salts (per cent)	Na_2CO_3 (per cent)	Other carbonates (per cent)	Chlorides (per cent)	Sulphates (per cent)	Calcium (per cent)
1	0—6 in.	0·24	In Traces only	0·116	0·018	0·131	0·016
2½	6—12 in.	0·30		0·130	0·037	0·131	0·080
3	1—2 ft.	0·34		0·072	0·037	0·098	*Nil*
4	2—3 ft.	0·58		0·072	0·037	0·361	0·066
5	3—4 ft.	0·20		0·130	0·018	0·049	0·016
6	4—5 ft.	0·22		0·086	0·018	0·107	0·008

The total salts gradually show a tendency to increase. The maximum concentration is at the 3rd foot. After that they get lowest. The predominant salt is sulphate. This salt more or less fluctuates in the same way as the line of total salts. The carbonates are next to it and then calcium salts. Magnesium is in traces. The study of exchangeable bases of the whole profile with capillary rise and *p*H values are given in Table VI.

TABLE VI

Results of different soil tests of sodiumized fine black soil

Serial No.	Depth of soil profile	Total bases milli-equiv. (per cent)	Per cent mono-valent bases to total bases	Capillary rise in cm. in 300 minutes		*p*H values in		$CaCO_3$ (per cent)
				In water	In NaCl	Distilled water	*N*-KCl solution	
1	0—6 in. . . .	26·84	20·16	2·10	10·9	9·14	7·28	11·95
2	6—12 in. . . .	24·33	22·97	1·70	8·8	9·59	7·28	13·75
3	1—2 ft. . . .	27·87	19·25	2·00	7·9	9·44	7·32	12·60
4	2—3 ft. . . .	25·12	15·44	2·00	11·2	9·45	7·04	11·20
5	3—4 ft. . . .	25·78	13·31	2·10	6·10	9·46	7·10	15·60
6	4—5 ft. . . .	23·71	20·55	1·70	6·1	9·48	7·44	14·00

The predominant base is replaceable calcium, constituting about 80 per cent of the total bases. The monovalent bases range from 13 per cent to as high as 22 per cent. The capillary rise is also very low while the *p*H values are above 9·0.

ALKALI PROFILE No. B

Another typical profile was examined from stiff alkali spot from deep soil area with the results given in Table VII.

TABLE VII

Results of water-soluble salts and other tests

Depth of profile	Total salts (per cent)	Na_2CO_3 (per cent)	$NaHCO_3$ (per cent)	Chlorides (per cent)	Sulphates (per cent)	*p*H values in		Per cent monovalent bases to total bases
						water (distilled)	*N*-KCl solution	
0—6 in. . .	0·21	0·084	0·066	0·018	0·024	10·09	7·68	40·76
1—2 ft. . .	0·52	*Nil*	0·231	0·055	0·207	10·08	7·57	52·81
2—3 ft. . .	0·47	*Nil*	0·199	0·018	0·237	9·98	7·74	53·57
3—4 ft. . .	0·41	0·042	0·221	0·018	0·121	10·08	7·78	77·33
4—5 ft. . .	0·41	0·084	0·177	0·028	0·098	9·76	7·80	53·42

The salts are comparatively more than the previous profile and have concentrated at the 2nd foot. The carbonate salts are very high. The *p*H values are nearly up to 10·0 while the per cent monovalent bases are found to the extent of 40 per cent increasing up to 77 per cent at the 3rd to 4th foot soil column.

ALKALI PROFILE No. C

Profile from an intensely stiff alkali patch was examined further and gave the results given in Table VIII.

TABLE VIII

Results of water-soluble salts and other tests

Depth of soil	Total salts (per cent)	Na_2CO_3 (per cent)	$NaHCO_3$ (per cent)	Chlorides (per cent)	Sulphates (per cent)	*p*H values in		Per cent monovolent bases to total bases
						Distilled water	*N*-KCl solution	
0—1 ft. . .	0·42	0·086	0·068	0·037	0·185	10·20	7·64	62·48
1—2 ft. . .	0·53	0·056	0·133	0·055	0·265	9·80	7·52	50·20
2—3 ft. . .	0·83	0·056	0·087	0·074	0·595	9·60	7·54	38·38
3—4 ft. . .	0·95	0·028	0·111	0·074	0·720	9·64	7·54	35·65
4—5 ft. . .	0·31	0·028	0·155	0·055	0·197	10·10	7·32	65·78

In this profile the salts are increasing up to 4th foot after which there is sudden fall. The predominant salt is sulphate throughout the profile unlike the previous profiles. The secondary salts are carbonates. Chlorides are in very low quantities. The *p*H values are about 10·0 while the top foot is highly sodium saturated.

Mechanical analysis of these damaged soils profiles indicated that the B horizon was coarser than the top horizon.

CLASSIFICATION OF DAMAGED SOILS

Mixed saline soils

The first type of profiles wherein we find mixed salts of calcium and sodium and which on leaching give characteristic low *p*H values like normal soils and fairly high percentage of divalent bases can be termed mixed saline soils.

Saline soils

The next type of profiles examined consist mostly of sodim salts only with *p*H values on the higher range than mixed saline soils. These represent accumulation of salts under high water-table and are not leached either naturally or artificially. These we will call saline soils.

Alkali and strong alkali soils

Further profiles are alkali types wherein leaching conditions are established. The total salts are thus low at the top. These are stiff soils containing varying degrees of alkalization from 20 to as high as 50 to 60 percent. We find *p*H values over 9·0 and sometimes up to 10·0 with carbonate salts in great predominance : we will call these alkali and strong alkali soils.

We have thus the following classes of the damaged soils :—

(*i*) Mixed saline soils

(*ii*) Saline soils

(*iii*) Alkali soils—Strong alakali soils

(*a*) (*b*)

Illustration.—A careful study was made in a Government area known as Experimental Salt Area stationed at Baramati commanded by Distributary 24-25. Nira Left Bank Canal. About 300 representative soil samples were collected and *p*H values, capillary rise and soluble salts were estimated (Fig. 6).

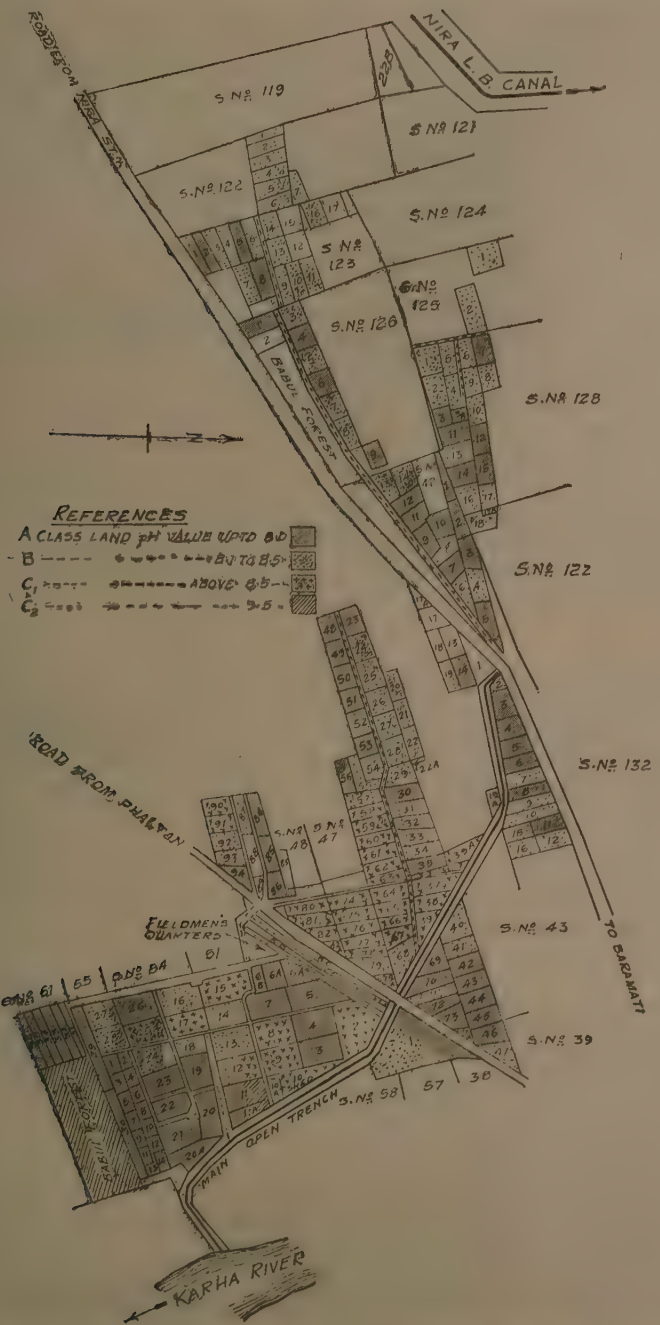


FIG. 6. **Experimental salt land, Baramati : Nira Left Bank Canal (scale 1 in.= 1,100 ft**

The whole area had salts varying from about 2 per cent to 4 per cent and more. It was noticed that on leaching certain plots, which had combined salts of calcium, magnesium and sodium, became free of excess of soluble salts rapidly and showed *p*H values up to 8·0. There were certain other plots in which soluble salts went down similarly but *p*H values were up to 8·5. Such gradations were noticed further, which is clearly illustrated in the figure. From the above description and the exhaustive tests of *p*H values, the damaged soils can be further classified as under :—

	Class of valuation
(1) High salts (which on leaching give low *p*H values up to 8·0) . .	A
(2) High salts (which on leaching give medium *p*H values up to 8·5) .	B
(3) High salts (without leaching) ; *p*H values above 8·5 . . .	C
(4) Low salts ; high *p*H values above 9·0	C_1
(5) High salts ; high *p*H values above 9·5	C_2

A reference may be made to the classification of damaged soils by De' Sigmond [1927] on the basis of sodium carbonate and total salts but the above classification has been found to be more suitable to Deccan conditions as actual tests of soil extract of even C_1 or C_2 types show very little alkalinity to Phenolphthalein. On the other hand, it is both rapid and precise to characterize alkali soils on the basis of *p*H values as mentioned above.

Practical application

If the damaged soil falls under class A, it means that the soil can be improved by drainage and simple leaching (either natural or artificial).

It is shown that sodiumization does not take place in such soil profiles due to sufficiently high concentrations of calcium and magnesium salts side by side with sodium salts.

To improve such soils the procedure will be as under :—

First step

Level the land and prepare it into small plots according to the slope of the lands and leach the excessive salts.

Second step

Take a test crop of *shalu jowar*. This is a sensitive crop and is a useful practical guide in this leaching process. If it is an 8—10 anna crop we can make out that the salt concentration is sufficiently lowered. This crop is then followed by a green manuring crop next season followed by sugarcane.

Plate LXVIII shows the first cane crop taken after leaching operations in a mixed saline soil of A type in survey No. 128 of Experimental Salt Area, Baramati. This shows how easy it is to reclaim such types of areas.

Improvements of damaged soils under class B will be on the same lines as A but this may require leaching over two seasons on accou t of comparative lower concentration of calcium and magnesium.

Improvement of C type is a different process because if leaching is done ke types A and B, the soils may turn into types C_1 or C_2 and will be still

difficult to reclaim. Improvement of these particular types require great skill which consists of addition of calcium fertilizers, sulphur, etc. along with farm yard manure. One such complete experiment is described.

Lysimeter experiment

Lysimeters of cement concrete were constructed in the midst of cane area in order to ensure proper 'cane atmosphere' to the cane grown in lysimeters. The lysimeters were rectangular in shape and measured 6 ft × 3 ft × 6 ft. Stiff alkali soil* was refilled in the lysimeter keeping the relative arrangements of layers the same as under natural conditions and with the same packing over a sloping bed of coarse sand 1 in. thick. This admitted drainage from the overlying soil layers. It was possible in lysimeters to carry out experiments under fully controlled conditions. Co 290 variety of cane was planted on 1 March 1938. The treatments given were as under :—

	Dose	Approximate cost (Rs.)
(1) Gypsum	3 tons per acre . . .	75
(2) $CaCO_3$	3 tons per acre . . .	70
(3) Sulphur	1/2 ton per acre . . .	70
(4) Blank or control	. . . . .	*Nil*

Farm yard manure at the rate of 15,000 lb. per acre was applied as a basal dose before planting. Three top dressings of ammonium sulphate and cake were given till the time of earthing up on the basis of 225 lb. of nitrogen.

Irrigation was given at an interval of 10 days and drainage was collected three days after irrigation was applied.

Results

The total water added was about 125 in. Gypsum treatment gave more drainage water than the rest of the treatments while sulphur stood second. This will be clear from Table IX.

TABLE IX

Drainage water received under different treatments

	Gypsum	Sulphur	$CaCO_3$	Blank
Drainage water in inches . . .	42·08	38·12	37·04	24·68
Percentage of drainage received to total water added.	29·64	26·86	26·12	17·36

*pH 9·0 ; soluble salts 0·55 per cent ; capillary rise 2·8 cm. in 300 minutes

The drainage water was tested for total soluble salts and *p*H values and it was noted that gypsum and sulphur removed larger quantities of salts than the rest. The *p*H values of drainage water showed it to be more alkaline during July to December than during the rest of the year (Fig. 7). This was

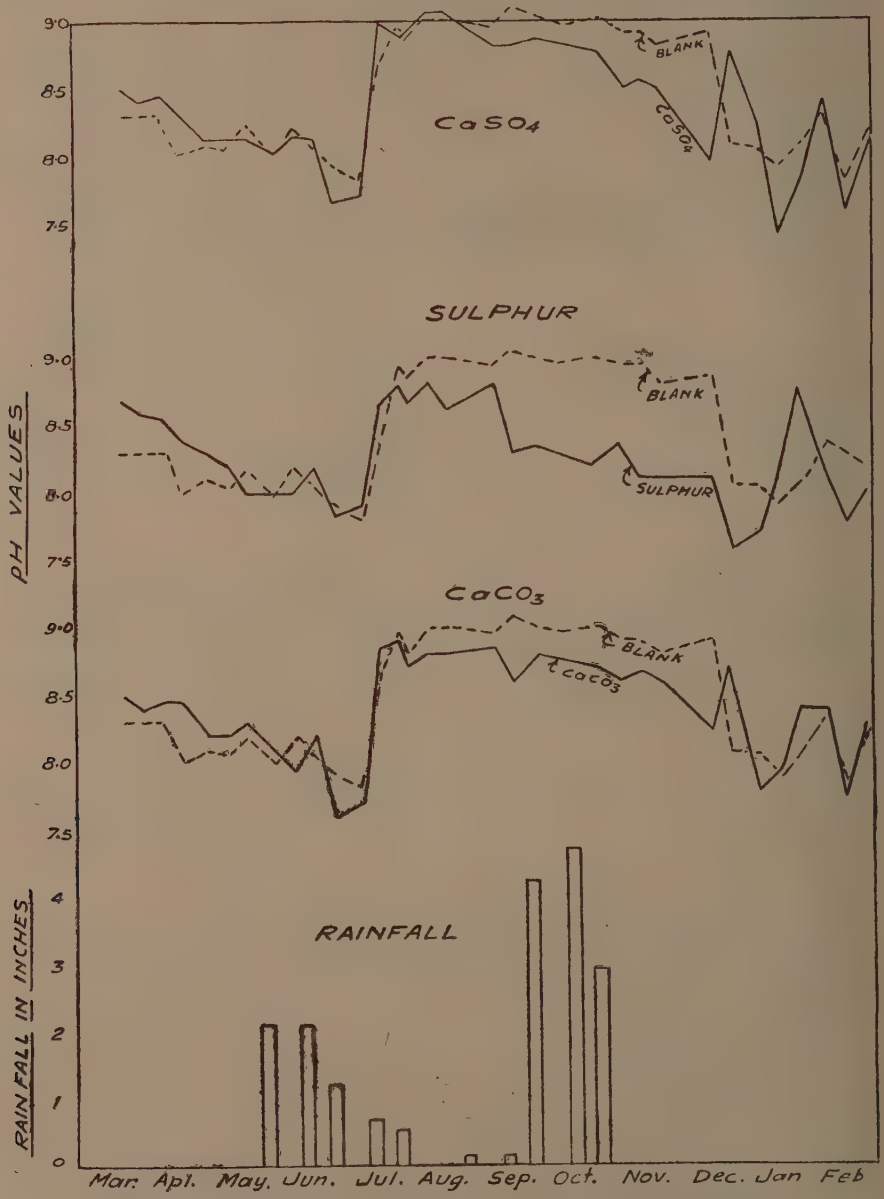


FIG. 7. Graph showing *p*H values of drainage waters in lysimeter

due to more dilution and consequent reduction in the conductivity of drainage water. The drainage water from the 'blank' was more alkaline than that from the treatments, but the quantity was much less. The crop was poor in the beginning but made a fairly good progress after earthing up. The total heights of cane under each treatment at the time of harvest and other relevant data are given in Table X.

TABLE X

Data of sugarcane harvested under different treatments

Treatment	Height of cane	Weight of cane in lb	Brix of juice	Purity of juice	Conductivity of juice
Sulphur . . .	5 ft. 9 in. .	30	17·87	82·8	8,500
$CaSO_4$	6 ft. 2 in. .	30	17·87	82·5	7,500
$CaCO_3$	5 ft. 8 in. .	25	17·87	78·4	9,000
Blank	5 ft. 2 in. .	10	16·87	78·2	9,000

The results show the growth and quality of cane under each treatment. Sulphur and $CaSO_4$ are outstanding from this point of view. Results of soil profiles examined after harvesting cane are given in Table XI.

TABLE XI

*pH values of residual soil at different depths**

Original soil *p*H	Depth (inches)	Sulphur *p*H	$CaSO_4$ *p*H	$CaCO_3$ *p*H	Blank *p*H
8·90	0—6	7·44	7·72	7·56	7·56
	6—12	7·36	8·20	7·68	7·94
9·00	12—18	7·76	7·98	7·98	*8·39*
	18—24	8·24	*8·24*	*8·54*	9·12
9·10	24—30	8·42	8·96	8·84	8·49
	30—36	8·00	9·52	9·24	8·60
9·00	36—42	8·14		9·49	9·49
	42—48	*7·70*	9·00	9·49	9·00

* Figures in italic indicate the depth to which improvement progressed

It is seen that sulphur treatment improved the whole profile. Gypsum and $CaCO_3$ affected improvement up to 24 in while in ' blank ' (with cane) the soil improved up to 18 in. only.

Field experiments

Plot scale experiments were laid out to study the behaviour of different varieties of cane in lands in process of reclamation.

Preparatory tillage and doses of manure were according to the standard practice. But planting was done on sides of ridges and soils were stirred every month till the time of earthing up. Several stools were collected and observations on conductivity, sucrose content and total solids were taken in certain cases from juice. The results are given in Table XII. The tendency is a fall in sucrose with increase in conductivity. This shows that we may get a little inferior gull from lands in process of reclamation.

Table XII

Results of conductivity and sucrose of different varieties tried in alkali soils

Serial No.	Variety	Conductivity of juice by Dionic water tester	Sucrose (per cent)
1	POJ 2878	2500	17·58
2	POJ 2878	3000	18·83
3	Co 290	5500	14·29
4	Co 290	4250	15·72
5	Pundia (local cane)	2500	17·87
6	HM 320	2500	16·27
7	HM 320	5000	16·49
8	EK 28	2800	17·77
9	EK 28	3500	17·96
10	POJ 2883	3500	15·77
11	POJ 2883	4500	15·93
12	Co 360	2500	17·94
13	Co 417	5500	14·89
14	Co 419	4700	15·23

Detailed results of field experiments on varieties from which the above observations were taken are given below :—

The experiment was carried out in replicates and on randomized basis and results were treated statistically.

	Pundia	POJ 2878 III	POJ 2883	EK 28	HM 320 II	Co 290 I
Mean yeild of 4 replicates (tons per acre)]	11·73	24·00	19·77	20·04	25·94	40·13
Difference	...	12·27	8·04	8·31	14·21	28·40

Significant figure 8·25 tons.

The results show that Co 290 stands first. Statistical treatment showed it to be significantly higher than Pundia ; POJ 2878 and HM 320 stand next in order.

Summary of results

The Deccan soils have four types of damaged soils excluding waterlogged areas.

(1) Mixed saline soils.
(2) Saline soils.
(3) Alkali and strong alkali soils.

These require different treatments for their improvement. The first type merely improves by leaching while the saline soils get alkaline either under natural or artificial conditions of leaching and require fertilizers for their improvement. Gypsum and sulphur in combination with farm yard manure have been found to be the best. As regards cropping Co 290 sugarcane variety has proved to be alkali resistant and is tried after Dhaincha (Sesbania Aculeata) green manuring with a basal dose of gypsum and farm yard manure.

There is a great field for use of gypsum as there are extensive deposits of this stuff in the Deccan.

Acknowledgement

The Author takes the opportunity of thanking Rao Saheb R. G. Islur, L.C.E., B.S.E. (I), Executive Engineer, Poona Irrigation and Research Dn., for permission to publish this Paper.

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STUDIES ON PHYSICO-CHEMICAL CHANGES IN BLACK COTTON SOIL DURING NITRIFICATION*

BY

MATA PRASAD

AND

N. K. PATWARDHAN

Royal Institute of Science, Bombay

(Received for publication on 27 December 1940)

THE black cotton soil, more commonly known as regur soil, from the Malwa plateau (e.g. field No. 45 at the Institute of Plant Industry, Indore, Central India, from which soil for the present investigation was collected) has a very high clay content and a high base exchange capacity. The nitrogen balance of the soil during summer and the nitrification of the added inorganic and organic nitrogen has been studied by Wad and coworkers [1936, 1937, 1938]. Very little attention, however, appears to have been paid to the study of the physico-chemical properties of this soil, following treatment with ammonium sulphate. It is probable that such a study may throw light on the question, whether the changes in the tilth of the soil during crop growth are in any way related to nitrification. In the present investigation an attempt has been made to study some properties of the black cotton soil as nitrification proceeds in the untreated soil and in soil treated with two different doses of ammonium sulphate.

Experimental

Material and methods

The technique used was essentially that described by Wad and Panse [1933]. Soil, evenly graded (Table I) by passing through 1 mm. sieve was uniformly filled in galvanized iron trays 12 ft. × 6 ft. × 2 ft., after moistening it with only about 1/3 of the total water added. The water content was then made-up-to about 26-27 per cent, as it has been found by Plymen and Bal [1925] that under these conditions soil shows the maximum nitrifying activity. The addition of water was done by means of a fine light jet.

The rates of the treatments given were (*i*) 25 lb. and (*ii*) 50 lb. of N-equivalent of ammonium sulphate (Merck's A. R. quality) per acre of 6 inch deep soil. The trays were arranged in blocks with treatments randomized in two well ventilated chambers with glass doors. Daily temperature and humidity changes occurring during the experimental period were recorded. The experiments were conducted for a period of four months, during the Bombay monsoon, at this institute. Samples were taken out five times, starting with the initial, with about four weeks' interval and there were four

* Part of a thesis submitted by the junior author and accepted by the Bombay University for the degree of Master of Science.

repetitions for each of the above ; this has been found necessary for the statistical examination of results. Thus $3 \times 5 \times 4$, that is, 60 trays were employed for the experiment.

Samples, 12 at a time, were removed on 11 August 1939 (initial), 12 September, 10 October, 13 November and 12 December. They were analysed (*a*) every month for ammoniacal nitrogen, nitrate nitrogen, organic carbon, C/N ratio and hygroscopic moisture ; and (*b*) every two months for base exchange capacity, total and individual replaceable bases, available phosporic acid (P_2O_5), aggregate analysis and resistance to water (structure coefficient).

Analytical methods

1. Ammoniacal nitrogen was determined by distilling 1 : 30 soil solution with MgO (5 gm.), absorbing the liberated ammonia gas in standard acid, and titrating back the remaining acid.

2. Nitrates were determined by the phenol-disulphonic-acid method.

3. C, N and C/N ratio were determined by Maclean-Robinson's method with slight modifications wherever necessary.

4. Hygroscopic moisture was determined by keeping the soil over 50 per cent humidity as done in Puri, Crowther and Keen's method.

5. Available P_2O_5 was determined in 1 per cent citric acid extracts followed by Pemberton's volumetric method.

6. Exchangeable calcium, magnesium, sodium and potassium and exchange capacity were determined by Puri's ammonium carbonate method, modified for calcareous soils [1935, 1936].

7. Mechanical analysis (of water dispersed and mechanically dispersed soil) was carried out by Bouyoucos' method [1934]. Structure coefficient (S. C.) as postulated by Russell [1938] was calculated from the relation—

$$\text{S. C.} = \frac{D - S}{D} \times 100$$

where D = per cent material $> 0{\cdot}05$ mm. as obtained by mechanical dispersion.

S = per cent material $> 0{\cdot}05$ mm. as obtained by water dispersion.

Results

The results of analysis of the graded soil used for this investigation are given in Table I.

Table I

	Unsieved portion				Sieved portion
	Plant residue	Rock fragments	Kankar 1·5—0·5 cm.	Murrum 0·5—0·15 cm.	0·1 cm.
Top loose earth 0—2 in.	0·2	0·4	2·1	9·2	88·1
Lower compact earth 2—6 in.	0·0	1·0	2·0	27·0	70·0
Average . . .	0·1	0·7	2·05	18·1	79·05

It will be seen from the above table that the soil formation processes are most active within two inches from the surface. The amount (about 90 per cent) of fine soil material (sieved portion) in this region is much greater than in the lower compact soil.

The results of complete chemical and physical analysis of the sieved soil used for the experiment are given in Table II. All the results are expressed on oven-dried basis.

TABLE II

Chemical and physical analysis of the sieved soil

(*i*) Gm. in 100 gm. of soil

Silica SiO_2	57·090
Al_2O_3	13·616
Fe_2O_3	8·674
CaO	3·633
MgO	2·897
K_2O	0·821
Na_2O	0·451
Loss on ignition	10·387
Total	97·569

Ratios—

SiO_2/Al_2O_3	4·193
SiO_2/R_2O_3	2·56

(*ii*) Mg. in 100 gm. of soil

Ammoniacal N	4·19
Nitrate N	0·6474
Total N	73·0
Organic carbon	499·0
Available P_2O_5	13·3
Available K_2O	0·4829

Ratio C/N = 10·10

(*iii*) Milli-equivalents per 100 gm. soil

Base exchange capacity	60·87
Total exchangeable bases	64·72
Exchangeable calcium	93·93 per cent of total bases
Exchangeable Mg.	5·359 per cent of total bases
Exchangeable K	0·3796 per cent of total bases
Exchangeable Na	0·3282 per cent of total bases

(*iv*)

Hygroscopic moisture (on fresh basis) . 5·9675 per cent

(*v*) Box constants

Apparent density	1·398 gm. per c.c.
Water holding capacity	46·71 per cent.
Specific gravity	2·231
Pore space	55·30 c.c. per 100 c.c.
Volume expansion	33·53 c.c. per 100 c.c.
Conductivity	$186·7 \times 10^{-6}$ r. o.
Total soluble salts (weight)	0·047 per cent

(*vi*) Mechanical analysis

(*a*) By Bouyoucos' method	(1·00-0·05)	(0·05-0·005)	(0·005-0·002)	<0·002
Aggregate (water dispersed)	74·2	21·4	2·4	2·0
Ultimate (mechanically dispersed)	9·9	35·7	6·7	47·7
		(0·5-0·05)	(0·05-0·005)	<0·005
(*b*) Pipette method (Puri's Nacl Dispersion method)		25·5	20·0	53·8

(*c*) Structure coefficient— $\frac{D-S}{D} = 0·7137$

The results of the periodical examination (expressed on oven-dried basis) are given in Table III. These are the averages of four determinations. They have been statistically examined by Fisher's analysis of variance and standard error for the results and their (marginal) averages, are also given.*

TABLE III

Nitrate nitrogen mg. per 100 gm. soil

(Average results)

	Average time						Standard error
	Initial	32 days	60 days	94 days	123 days	Average	
Control	2·58	2·06	2·97	6·22	5·21	3·81	
25 lb. N	5·25	4·05	5·20	8·48	8·71	6·34	±0·3448
50 lb. N	7·59	10·94	7·77	10·27	10·06	9·32	
Standard error = ±0·7712							
Average	5·14	5·68	5·32	8·32	7·99		

Standard error = ±0·4453

It will be seen from the above analysis that the untreated soil and that treated with 25 lb. of nitrogen do not show any increase in the nitrate nitrogen up to 60 days, and the rise after this period remains practically constant. But with 50 lb. the nitrate nitrogen increases in the first four weeks and then falls during the next month; it again rises after eight weeks and remains steady.

* For convenience of presentation the data in the tables are given only up to two places of decimal although in the statistical computation figures up to four places of decimal were used.

If the averages are considered, it is found that, on the whole, in all the three cases, the nitrate nitrogen remains steady up to 60 days ; it reaches its highest value after 94 days and remains steady in the following month. The sudden increase in the 50 lb. N treatment after 32 days can be explained as an interaction.

During all the five stages there is, on the whole, a tendency for an increase in the nitrification in 25 lb. N treatment which is greater than the control, while that treated with the double dose is significantly greater than the one with the single dose. It will also be seen that the single dose shows a higher production of nitrate nitrogen than the control at all stages excepting the second. The 50 lb. application produces significantly more nitrates than the 25 lb. one, up to 8 weeks only, although its nitrate contents are higher than the control in the last two stages. It will be noted that the nitrate nitrogen obtained by analysis is greater than the added N, which is about 2 mg. in the case of 25 lb. N and 4 mg. in the case of 50 lb. N per 100 grams of the soil. This shows that in addition to the nitrogen added, the soil nitrogen also undergoes nitrification. Similar observations have been made by Yuen and Boden [1937] who found that the rate of nitrification is variable and the process does not necessarily occur like a quantitative reaction, the increase in the nitrate nitrogen being sometimes greater than the equivalent of nitrogen added.

TABLE IV

Ammoniacal nitrogen mg. per 100 gm. soil

(Average results)

	Average time						
	Initial	32 days	60 days	94 days	123 days	Average	
Control	7·37	4·24	3·39	2·54	4·34	4·35	Standard error ±0·29
25 lb. N	6·57	3·85	2·64	3·00	4·34	4·03	
50 lb. N	5·39	3·77	3·06	3·54	4·10	3·97	
Standard error = ±0·62							
Average	6·44	3·95	2·94	2·99	4·26		

Standard error = ±0·38

It will be seen from Table IV that in the treated soils and the control the ammoniacal nitrogen starts decreasing during the first 60 days. In the control and the 25 lb. N treatment it has already decreased significantly in the first 32 days, whereas in the case of 50 lb. N treatment it slowly decreases till this decrease reaches its significance in 60 days. Thus, it can be concluded that the nitrification is rapid in the control and the 25 lb. N treatment. The ammoniacal nitrogen content remains constant later on in the two treated samples but the control shows a slightly significant increase after 94 days.

If these results are examined together it can be seen that there is an abundance of ammoniacal nitrogen in the initial stages but it decreases in

the next four weeks. Then it remains steady for 94 days and afterwards shows an increase. But it will be seen that the ammonia contents of both the treated and the untreated soil are small at all stages.

It will be further seen that there is not much difference in the ammoniacal nitrogen content due to the differences in the treatment. Only in the initial stage the control shows a significant increase over the 50 lb. N treatment. At other stages there are no significant differences. This can also be seen to be true from the averages of the results.

TABLE V

Organic carbon mg. per 100 gm. soil

(Average results)

	Average time						
	Initial	32 days	60 days	94 days	123 days	Average	
Control	412·8	512·1	633·1	577·8	564·1	541·9	Standard error ±12·45
25 lb. N.	342·8	553·5	627·4	617·7	522·9	532·8	
50 lb. N.	482·7	582·2	640·3	591·5	491·8	557·7	
Standard error = ±26·91							
Average	412·8	549·2	633·6	598·9	526·2		
Standard error = ±16·09							

In all cases the organic carbon increases in the first 32 days ; in the next 28 days there is a significant increase over the last stage in the case of the control only. In the next 34 days the carbon contents are steady in all the three cases. But during the last 30 days although the carbon content in the control and 25 lb. N treatment remains steady, with slight fluctuation, it decreases significantly in the double treatment.

In general, it will be seen that there is a tendency for the carbon content to increase in all the three cases during the first 60 days of the experiment. Later on it remains steady for the next 34 days.

There are no differences in the carbon content due to the differences in the treatment in all the five stages. However, it will be noted that, in the initial stage, there is some difference (not significant) in the three cases. The general average also shows a fair constancy of the carbon content in the different treatments.

It can be seen from Table VI that in the case of control, the ratio is steady in the beginning, increases in the next 28 days and remains steady later on. However, in the case of the 25 lb. N treated samples the increase is significant in the first 32 days and in the next 28 days, whereas in the 50 lb. N treatment there is a significant increase only in the first 32 days. Later on, the ratio in the two treated sampels remains constant with insignificant variations, although a tendency for decrease is seen.

TABLE VI

C/N ratio

(Average results)

	Average time						Standard error
	Initial	32 days	60 days	94 days	123 days	Average	
Control	4·82	5·33	6·54	6·28	6·75	5·96	
25 lb. N.	3·57	5·98	7·21	6·56	5·94	5·77	±0·17
50 lb. N.	5·29	6·45	6·38	6·16	5·61	5·99	
Standard error = ±0·37							
Average	4·66	5·79	6·71	6·36	6·03		

Standard error = ±0·22

The results as a whole show that the ratio increases during the first 60 days, remains steady during the next 34 days, and starts decreasing later on.

No changes take place (in the initial and the subsequent stages) due to different treatments. In the initial stage the ratio in the case of 50 lb. N treatment and the control is greater than the 25 lb. N treatment. This is in accord with the organic carbon content in the corresponding stage. In the next stage, however, the ratio in the 50 lb. N treatment is significantly greater than the control. The general results show no significant increase or decrease in the ratio due to the different treatments. Since the C/N ratio shows similar variations as carbon it may be inferred that there may not be any changes in the actual nitrogen content of the soil during the experimental period. Russell has shown that this nitrogen cannot increase by itself ; and increase is possible only when some carbonaceous matter is added along with the nitrifying agent to the soil. The gain in nitrogen, under these circumstances, is only 1/10 of that of the carbon. Only nitrogeneous fertilizers such as $(NH_4)_2SO_4$ do not add any nitrogen to the soil beyond that which corresponds to the carbon added, if any, to the soil by the stubble.

TABLE VII

Hygroscopic moisture per 100 gm. soil

(Average results)

	Average time						Standard error
	Initial	32 days	60 days	94 days	123 days	Average	
Control	5·53	6·26	8·02	6·85	6·23	6·58	
25 lb. N.	5·64	6·46	8·01	6·86	6·24	6·64	±0·03
50 lb. N.	5·47	6·37	8·08	6·86	6·31	6·62	
Standard error = ±0·07							
Average	5·55	6·36	8·04	6·86	6·26		

Standard error = ±0·043

The changes in the hygroscopic moisture in the different stages are highly significant. It can be seen that in all the three cases the hygroscopic moisture increases in the first 60 days, reaches a maximum value and then decreases. The values reached at the end of 123 days are nearly the same as those after 32 days. Hence it may be concluded that the decrease in the available water during the first two months is comparatively more rapid than its increase during the next two months. Assuming that the hygroscopic moisture is proportional to colloid content the marked increase in the latter at the end of the eighth week perhaps causes a lack of aeration which leads to the formation of an algal film which was observed in all cases [Wad and Panse, 1933].

During all the stages it is found that there are no significant differences in the hygroscopic moisture, and hence, in the colloidal content due to the addition of manure. However, it may be mentioned that on the whole the colloid contents are numerically higher in the case of the 25 lb. N treatment than 50 lb. N treatment, which is in turn higher than the control, although these differences are insignificant.

TABLE VIII

Available P_2O_5 mg. per 100 gm. soil

(Average results)

	Average time				
	Initial	60 days	123 days	Average	
Control	13·69	13·03	10·24	12·30	Standard error ±0·43
25 lb. N	11·49	14·81	10·35	12·24	
50 lb. N	13·78	15·51	12·02	13·77	
Standard error= ±0·74					
Average	13·01	14·45	10·85		
Standard error= ±0·43					

It will be seen that the available P_2O_5 content in the case of the control and the 50 lb. N treatment remains steady in the first 60 days although there is a tendency to increase in the latter case (not a significant one). But in the case of the 25 lb. N treatment there is a more significant increase in this period. In the next 60 days the available P_2O_5 content significantly decreases in all the three treatments and the contents on the 123rd day are less than in the initial stage. The average results show that the P_2O_5 content increases in the first 60 days and decreases evenly during the subsequent two months.

It will also be seen that in the initial stage the 50 lb. N treatment and the control contain the same amount of available P_2O_5 and this is significantly greater than the value for the 25 lb. N treatment. But after 60 days the 50 lb. N treated samples have significantly more P_2O_5 than the control, the 25 lb. N treatment being midway, though the differences are insignificant. In the next 63 days the 25 lb. N and the control have practically the same available P_2O_5 whereas 50 lb. N treatment has more. This is also clear from the average results which show that the 50 lb. N treatment is significantly superior to the control and the 25 lb. N treatment as regards the available P_2O_5 contents.

TABLE IX

Base exchange capacity (m.e/100 gm. soil)

(Average results)

	Average time				
	Initial	60 days	123 days	Average	
Control	56·20	57·21	55·61	56·34	Standard error ±0·24
25 lb. N.	56·47	57·48	56·11	56·69	
50 lb. N	55·76	57·57	55·92	56·41	
Standard error= ±0·42					
Average	56·14	57·42	55·88		

Standard error= ±0·24

The base exchange capacity increases in the first 60 days only in the case of the soil treated with 50 lb. N. In the other two cases the increase is not significant though there is a slight rise. But in the following 63 days the exchange capacity decreases significantly in all the cases. The average results show that the exchange capacity increases in the first two months and decreases in the next two, attaining the original level once again. Thus it may be surmised that in the case of the control and 25 lb. N treatment the maximum exchange capacity might have been attained sometime between 40 and 80 days from the start.

There are no differences in the exchange capacity at any stage due to the differences in the treatment. In general the control as also the treated soils behaves similarly as regards the exchange capacity.

TABLE X

Total exchangeable bases (m. e. per 100 gm. soil)

(Average results)

	Average time				
	Initial	60 days	123 days	Average	
Control	56·17	63·68	59·58	59·81	Standard error ±0·33
25 lb. N	59·77	61·30	60·91	60·65	
50 lb. N	59·24	60·87	60·78	60·29	
Standard error= ±0·57					
Average	58·39	61·95	60·42		

Standard error= ±0·33

The total exchangeable bases in the control increase during the first 60 days but in the case of the treated samples the increase is not significant though there is undoubtedly a tendency to increase. It may be that the interaction in this case is rapid and the maximum was reached before 60 days, that is earlier than in the case of the control. In the next two months the treated samples show a decrease which is very marked in the case of the control. Further, the average results show that the total exchangeable bases increase in amount during the first 60 days and decrease in the next 60 days. The amount of the bases on the 123rd day is greater than that at the start in all cases (distinctly significant in the case of control only).

The results for the treatments present a very interesting example of interaction, for in the initial stage both the 25 lb. and 50 lb. N treated samples have a markedly higher base content than the control but after 60 days the total base content of the control is greater than that of the treated samples. In the last stage, however, these values are almost identical irrespective of the treatments. It is observed that the value for the total exchangeable bases are generally higher than the base exchange capacity of corresponding soil sample. It must be pointed out in this connection that the individual replaceable bases were determined on the soil samples not freed from soluble salts either by electrodialysis or by leaching with water. It is therefore possible that the differences might be due to this cause, but in view of the somewhat large differences observed (up to 11 per cent), there might be other unknown factors for the discrepancies.

It is seen that in all the three cases the exchangeable calcium increases in the first 60 days and then remains almost steady. Same conclusion can be drawn from the average results. The treatments amongst themselves show a peculiar behaviour for at the initial stage the exchangeable calcium of the 25 lb. and 50 lb. N treatments is significantly greater than that of the control

but in the subsequent stages it has almost the same value in all three cases In general it can be seen that the soil receiving the double treatment is definitely richer in exchangeable calcium than the control. That receiving the single treatment behaves almost similarly as the other treated soil. No determinations were made of soluble calcium, *p*H, and bicarbonate at the different stages. In the absence of these data, therefore, it is difficult to interpret the changes in the exchangeable calcium resulting from the treatments, for the calcium analytically determined need not necessarily be present in the exchangeable form.

TABLE XI

Exchangeable calcium (m. e. per 100 gm. soil)

(Average results)

	Average time				
	Initial	60 days	123 days	Average	
Control	91·17	93·90	94·11	93·06	Standard error ±0·20
25 lb. N	92·29	94·23	94·46	93·66	
50 lb. N	92·95	94·12	94·43	93·83	
Standard error= ±0·35					
Average	92·14	94·08	94·33		

Standard error= ±0·20.

TABLE XII

Exchangeable magnesium (m. e. per 100 gm. soil)

(Average results)

	Average time				
	Initial	60 days	123 days	Average	
Control	7·79	5·29	5·33	6·14	Standard error ±0·19
25 lb. N	7·10	5·11	5·14	5·78	
50 lb. N	6·42	5·45	5·21	5·69	
Standard error= ±0·33					
Average	7·1076	5·288	5·22		

Standard error= ±0·19

With the control and the 25 lb. N treatment there is a significant decrease in the exchangeable magnesium during the first 60 days whereas in the case of the 50 lb. N treatment the decrease is not very significant. The exchangeable magnesium remains fairly steady during the next 63 days in all three cases.

It will also be seen that there are no significant differences in the content of exchangeable magnesium in the different stages owing to the differences in the treatment, except in the initial stage where the exchangeable magnesium of the control is significantly greater than that of the treatments, specially, the double treatment. This may be due to the fact that the magnesium in the two latter cases does not exist in the exchangeable form. It is also probable that the exchangeable magnesium contents of the 50 lb. N treatment are not correctly determined, for no differences are noticed due to the differences in the treatment in the average results.

TABLE XIII

Exchangeable potassium (m. e. per 100 gm. soil)

(Average results)

	Average time				
	Initial	60 days	123 days	Average	
Control	0·76	0·57	0·40	0·58	Standard error ±0·03
25 lb. N	0·40	0·45	0·28	0·38	
50 lb. N	0·32	0·23	0·20	0·27	
Standard error= ±0·05					
Average	0·5202	0·4203	0·29		

Standard error= ±0·03

The treated and the untreated soils behave differently with respect to the exchangeable potassium content. While in the case of the control the exchangeable potassium shows a continual decrease, there is no significant change in the treated samples. However, in the 25 lb. N treated soil there is an indication of the approach of a significant decrease during the last 63 days. The average results, however, show a significant decrease with time.

Differences in the exchangeable potassium due to the differences in the treatment are significant in the initial stage. The potassium contents in both the 25 lb. N and 50 lb. N treatments are significantly less than that in the control. At the next two stages the exchangeable potassium significantly

decreases in the 50 lb. N treatment as compared with the control but no significant change is noted in the 25 lb. N treated soil. But the average results show that the significant effect of treatment is to cause a decrease in the potassium content.

In this case the blocks are also significant which shows that the random distribution of the trays has taken part in bringing about the above changes.

TABLE XIV

Exchangeable sodium (m. e. per 100 gm. soil)

(Average results)

	Average time				
	Initial	60 days	123 days	Average	
Control	0·24	0·21	0·14	0·20	Standard error ±0·008
25 lb. N	0·18	0·19	0·12	0·17	
50 lb. N	0·21	0·20	0·15	0·19	
Standard error= ±0·015					
Average	0·21	0·20	0·14		

Standard error= ±0·008

All the three sets of soils behave similarly. In the first 60 days the exchangeable sodium remains constant and there is a significant decrease in the next 63 days.

There are no significant differences due to the differences in the treatment. Only at the first stage of the control the exchangeable sodium is significantly greater than that of the 25 lb. N treated soil. In the other two stages it has the same value for all three sets of soil.

In all three cases there are no changes in the structure coefficient which determines the soil fertility at any period.

CONCLUSIONS AND SUMMARY

The results of the present investigation show that many of the improvements in the soil properties take place during the first two months after the treatment. Thus, the amount of nitrate which is supposed to have an adverse effect on soil is least in this period and increases only after three months from the start. The ammoniacal nitrogen is in equilibrium with the nitrate nitrogen during this period. The organic carbon also increases during the

first two months as a result of the formation of algae. Afterwards it starts decreasing ; this decrease may be due to an increase in the nitrifying activities probably at the cost of the algae.

TABLE XV

Structure coefficient

(Average results)

	Average time				
	Initial	60 days	123 days	Average	
Control	0·76	0·78	0·76	0·77	Standard error ±0·006
25 lb. N	0·80	0·77	0·80	0·79	
50 lb. N	0·75	0·80	0·78	0·77	
Standard error= ±0·01					
Average	0·77	0·78	0·78		
Standard error= ±0·006					

The C/N ratio remains fairly steady throughout the experiment, though there is an increase in the fertility of the soil. The available P_2O_5 content does not seem to have any correlation with the nitrate nitrogen.

The base exchange capacity, the total exchangeable bases and the exchangeable calcium increase in the first two months and there is a decrease in the next two months. This fact indicates the necessity of determining, correlations, if any, existing between these quantities and opens a new line for such work. Replaceable sodium and potassium decrease both with the treatment and time while exchangeable magnesium decreases only with time and not with the treatment.

The hygroscopic moisture increases in the first two months which shows that the colloidal matter of the soil increases during this period. But the treatment with ammonium sulphate does not show any changes in the clay content of the soil. Also the structure coefficients at various periods during the experiment do not show any changes even at the higher rates of treatment. This shows that the application of ammonium sulphate causes no deterioration in the soil structure of a permanent nature as far as the crop-season is concerned.

In conclusion it may be said that the application of ammonium sulphate in doses used in this experiment increases the soil fertility and productivity. This important conclusion is in conformity with the observation (Annual Reports I. P. I.), that the black cotton soil yields better crop when small

doses of ammonium sulphate (e.g. as used in this investigation) are added. In the two doses given in this work the soil properties are altered only in the first two months of experiment. This period of two months is the usual duration for the vigorous growth of a plant. The results point to the utility of the application of the fertilizer in the appropriate dosage to cause maximum beneficial effects during the period.

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RESPIRATION STUDIES OF THE ALPHONSO MANGO

BY

D. V. KARMARKAR, M.Sc., PH.D., A.I.I.Sc.

AND

B. M. JOSHI, M.Sc.

Cold Storage Research Scheme, Kirkee

(Received for publication on 27 December 1940)

(With Plate LXIX and two text-figures)

DURING the course of the cold storage investigations on mangoes [Cheema, Karmarkar and Joshi, 1939] it was observed that the Alphonso mango of 'B' stage of maturity (green and mature) was the best keeper. The most suitable range of temperature for storing the fruit was between 45° and 48°F. and the fruit ripened satisfactorily afterwards when removed to 68°F. Ripe yellow fruit (eating maturity) became chilled and turned brown at 52°F. and lower temperatures. Green fruit became chilled at temperatures lower than 45°F. and did not ripen properly when subsequently exposed to a higher temperature. Further experiments on the chilling of ripe Alphonso mango fruit showed that the browning occurring in storage at 52°F. could be prevented by keeping the fruit under partial vacuum. The respiratory activity is closely connected with the metabolic processes and it appeared that a study of the changes in the rate of respiration would throw light upon the nature of the physiological changes taking place in the fruit in storage. Experiments, therefore, were undertaken to determine the rate of respiration of the Alphonso mango under different storage conditions.

EXPERIMENTAL METHOD

The intensity of respiration was measured by the amount of carbon dioxide produced by the fruit sample kept in a closed container. A wide mouthed (4 in. diameter) glass jar of about 2500 c.c. capacity was used as the respiration chamber (Plate LXIX). The jar was closed by means of a tight-fitting rubber cork. A separating funnel and a bent glass tube were fitted into the cork. To the lower end of the funnel tube was attached a piece of rubber tubing reaching the bottom of the jar. Another piece of rubber tubing, provided with a pinch-cock, was attached to the outer end of the bent glass tube so that it could be connected either to a manometer during the respiration experiment or when required, to the inlet tube of the gas analysis apparatus.

The fruit sample was placed on a wire gauze platform about two inches above the bottom of the jar in order that the fruits did not come in direct contact with the water run into the jar through the separating funnel for displacement of the gas sample for analysis. The gas analysis was made at 68°F. using the Orsat's gas analysis apparatus. The percentages of both carbon dioxide and oxygen were determined. The change in pressure occurring in the respiration jar was noted and the necessary correction was made in

calculating the volumes of carbon dioxide and oxygen. The quantity of air available for respiration in the closed jar was the total volume of the jar minus the volume of the fruits. As the specific gravity of the mango is nearly unity, the volume of the fruits in c.c. was approximately equal to their weight in gm. The rate of respiration has been expressed as the volume in c.c. of carbon dioxide at 68°F. and 710 mm. (atmospheric) pressure, produced by 100 gm. of fruit in 24 hours.

The fruit used in these experiments was obtained from Ratnagiri and was in transit for two days. The temperature of the cold rooms in which the rate of respiration was determined showed a variation of ± 1°F.

Results

The course of respiration during ripening at 68°F.

Five samples, each consisting of three fruits of 'B' stage of maturity, were used. Cheema and Dani [1934] have defined this stage of maturity as the condition of fruit during development on the tree when the shoulders outgrow the stem-end and the colour is oil-green. The period of the respiration experiment was four hours and, after the experiment, the fruits were removed from the jars and kept in open trays. The respiratory activity of these five samples was determined every day until they became ripe. The data obtained are shown in Table I and are also represented graphically in Fig. 1. The values obtained for the initial rate of respiration at 68°F. showed

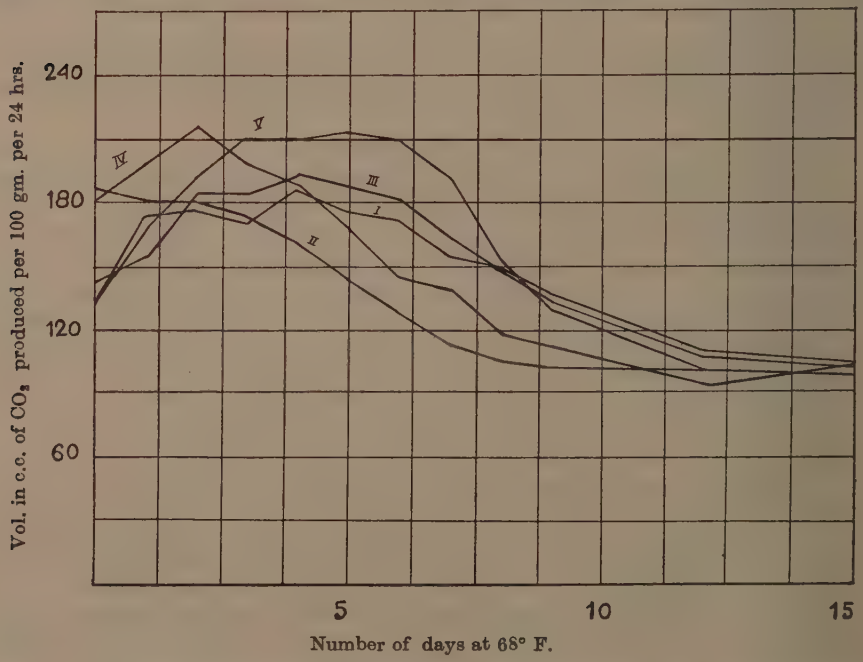
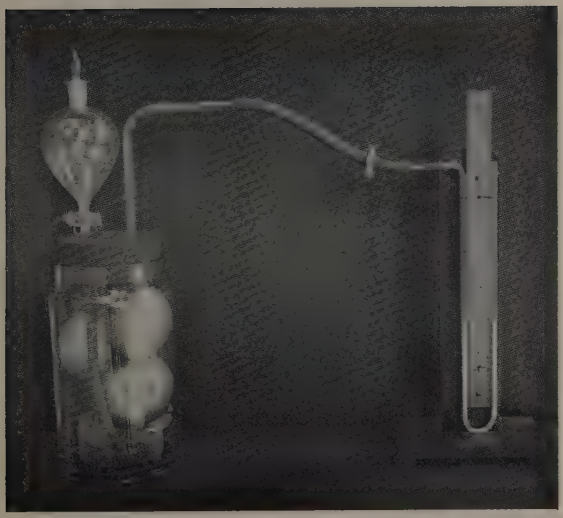


Fig. 1. The changes in the rate of respiration of green Alphonso fruit during ripening time at 68° F.



Apparatus used for the determination of respiratory activity

Table I

Changes in the respiratory activity during ripening at 68°F.

Number of days at 68°F.	Stage of ripening	Sample I		Sample II		Sample III		Sample IV		Sample V	
		CO_2 per 100 gm. per 24 hours c.c.	Respiratory quotient	CO_2 per 100 gm. per 24 hours c.c.	Respiratory quotient	CO_2 per 100 gm. per 24 hours c.c.	Respiratory quotient	CO_2 per 100 gm. per 24 hours c.c.	Respiratory quotient	CO_2 per 100 gm. per 24 hours c. c.	Respiratory quotient
0	...	134	1·084	186	1·069	142	1·012	179	1·076	134	1·067
1	Green and	175	1·097	180	1·032	153	1·058	198	1·075	167	1·042
2	hard	177	1·120	181	1·069	183	1·105	215	1·096	191	1·089
3	Slightly	172	1·108	173	1·098	184	1·110	197	1·073	210	1·126
4	changing	187	1·156	163	1·113	192	1·104	190	1·166	210	1·104
5	colour	176	1·193	143	1·086	187	1·160	167	1·137	213	1·179
6	...	175	1·199	130	1·024	181	1·134	148	1·127	209	1·185
7	Turning	157	1·164	114	0·944	161	1·130	141	1·066	192	1·172
8	Yellow	150	1·126	105	0·913	147	1·103	119	1·018	154	1·111
9	...	139	1·076	103	0·879	131	1·010	113	0·985	128	1·047
12	Ripe	111	0·914	102	0·841	104	0·866	97	0·856	102	0·902
15	Yellow	106	0·840	96	0·808	103	0·882	106	0·898	...	...
24	Over-ripe	...	...	...	...	100	0·825	101	0·767	101	0·845

Table II

Changes in acidity during ripening at 68°F.

Stage of ripening	Percentage of acidity in terms of malic acid on fresh weight basis
Green and hard	2·15
Slight change of colour	1·50
Turning yellow	0·29
Yellow	0·19
Fully ripe	0·18
Over-ripe	0·16
Ripened at 68°F. after storage for 30 days at 45°F. (fully ripe)	0·89
Ripened at 68°F. after storage for 40 days at 45°F. (fully ripe)	0·80

The percentage of acids thus decreased with the rate of respiration. The respiratory quotient (Table I) was only slightly greater than unity in the beginning, but it increased appreciably when the fruits commenced to turn yellow. This increase in the values of the respiratory quotient indicated that a part of the substrate for respiration consisted of the acids which became depleted during ripening. The value of the respiratory quotient decreased when the fruits were ripe and in the end became less than unity. Singh, Sheshagiri and Gupta [1937, 2] obtained values of the respiratory quotient which were less than unity and inferred that the nature of the respiration substrate in mangoes was a mixture of fats and carbohydrates. Wardlaw and Leonard [1940] have, however, shown the importance of the internal concentrations of carbon dioxide and oxygen in the fruit in studying the changes in the rate of respiration as determined by the amounts of carbon dioxide liberated at the surface and oxygen consumed.

Relation of the respiratory quotient to the freshness of fruit

The fruit used in the above experiments was obtained from Ratnagiri and was in transit for two days from that place to the Experimental Station at Ganeshkhind, Poona. The data given in Table I show that there was a rapid rise in the rate of respiration when the samples were kept at 68°F. It can be assumed that the rate of respiration was much lower at the time of picking. Fresh Alphonso fruit of 'B' stage of maturity was also obtained locally and the rate of respiration and the respiratory quotient were determined at 68°F. It was observed that not only was the rate of respiration very low (values between 40 c.c. and 80 c.c. were obtained) but the respiratory quotient of the local fresh fruit was less than unity (0·8 to 0·9) in all the samples. On storing the fruit at 68°F., the rate of respiration increased in two days and the respiratory quotient became greater than unity.

The less-than-unity values of the respiratory quotient indicate that a relatively larger volume of oxygen was consumed in the process of respiration than the volume of carbon dioxide produced. The manometer attached to the respiration jar consequently showed a depression in pressure. The respiratory quotient of the fruit kept for two days at 68°F. became greater than unity and the manometer showed a small increase in pressure. The depression in the pressure of the respiration jar could, therefore, be employed as a test of the degree of freshness of fruit. The respiration process has been utilized by Harvey and Rygg [1936] to indicate the vitality of citrus fruit in relation to its keeping quality.

Rrelation of temperature to the rate of respiration

Determinations of the rate of respiration were made at temperatures of 68°, 52°, 48°, 40° and 35°F. Two samples of green fruit were obtained and the same samples were used for determining the rate of respiration at the above temperatures. As mentioned above there was a considerable variation among individual samples and it was thought that using the same samples at different temperatures would give more reliable data than using separate samples at each temperature. First the rate was determined at 68°F. and then the samples were removed to 52°F. and the other temperatures. At

each temperature, the samples were kept for a day so that the fruits attained the temperature of the storage chamber. The results given in Table III showed that the rate of respiration decreased with temperature. There was, however, a marked decrease between 52° and 48°F.

TABLE III

The rate of respiration at different temperatures

Temperature	Volume of carbon dioxide in c.c. per 100 gm. per 24 hours	
	Sample I	Sample II
68°F.	142	173
52° F.	78	92
48°F.	43	47
40°F.	22	26
35°F.	17	20

RESPIRATION DURING STORAGE AT 48°F. AND AFTER REMOVAL TO 68°F.

The rates of respiration of three samples of 'B' stage fruit were first determined at 68°F. after which the samples were removed and kept at a storage temperature of 48°F. The rate was again determined at intervals during storage at 48°F. One of the samples was removed to 68°F. after thirty-five days of storage at 48°F. and another after forty-one days. The rates of respiration of these two samples were then determined at 68°F. at different stages of ripening. The data are given in Table IV.

The data show that, under storage at 48°F., the rate of respiration declined in the first fortnight, but afterwards remained steady up to the end of the storage period. The rates of respiration at 68°F. of green fruit removed from 48°F., after storage for thirty-five and forty-one days at that temperature (samples II and III), were lower than the initial rates of fresh fruit stored at that temperature, i.e. 68°F. It was noted that the rate of respiration did not decrease during ripening of the cold-stored fruit as in the case of fresh fruit ripened at 68°F. (Table I). The percentage of acidity (Table II) in the fully ripe fruit ripened after cold storage also remained higher than that in the fresh fruit ripened at 68°F.

THE EFFECT OF CHILLING ON RESPIRATION

The green Alphonso mango is chilled at temperatures lower than 45°F. and the fruit fails to ripen when subsequently removed to higher temperatures. It has also been observed that ripe yellow fruit is chilled at 52°F. and lower temperatures and the typical bright yellow colour turns brown. Two samples of green fruit were kept at 35°F. and three samples of ripe fruit at 52°F. The rates of respiration of these samples were determined at intervals. The

results are given in Table V. It can be observed that chilling did not appear to have any marked effect on the rate of respiration. At 52°F. the rate showed an increase after two weeks when the fruit became dark brown and rotting commenced.

TABLE IV

The rate of respiration during storage at 48°F. and after removal to 68°F.

	Volume in c.c. of carbon dioxide per 100 gm. per 24 hours		
	Sample I	Sample II	Sample III
Initial at 68°F.	252	218	194
Stored at 48°F.			
Number of days of storage at 48°F.—			
3	64	58	56
8	47	49	47
14	39	42	39
20	36	40	37
25	38	40	38
30	37	39	37
35	..	39	41
		Removed to 68°F.	
41	..	..	39
			Removed to 68°F.
Ripened at 68° F. after cold storage			
Number of days after removal to 68°F.—	Stage of ripening		
2	Green	195	164
6	Changing colour	176	172
9	Yellow	160	165
13	Fully ripe	171	159
17	..	170	..

TABLE V

The effect of chilling injury on the rate of respiration

35°F.			52°F.			
Number of days of storage	Volume in c.c. of carbon dioxide per 100 gm. per 24 hours		Number of days of storage	Volume in c.c. of carbon dioxide per 100 gm. per 24 hours		
	Sample I	Sample II		Sample I	Sample II	Sample III
0	20	17	0	79	79	73
8	20	20	2	67	70	69
18	23	20	4	66	76	74
			6	58	65	66
			9	65	72	68
			15	63	81	80
			22	73	85	86

RESPIRATION UNDER PARTIAL VACUUM

In their studies on the chilling of mangoes, the authors observed that the browning of ripe yellow fruit was prevented by storage under partial vacuum under which conditions the colour remained unaffected for a month at 52°F. The effect of partial vacuum on the rate of respiration was, therefore, investigated. The amounts of carbon dioxide produced by two samples of green fruit in four hours at 68°F. were first determined. The samples were then removed from the respiration jars and kept in a vacuum of 640 mm. of mercury and then put back into the respiration jars. The amounts of carbon dioxide produced by the samples in an equal period at 68°F. were again determined. Before vacuum, the percentages of carbon dioxide produced in the two jars were 5·0 and 7·2 and after vacuum the percentages of carbon dioxide were 5·4 and 7·6 respectively. It may, therefore, be assumed that subjecting the fruit to partial vacuum for a short time did not produce any effect on the rate of respiration.

The respiratory activity of fruit kept continuously under partial vacuum at 68°F. was also determined. The sample was kept in a vacuum desiccator which served as the respiration chamber. The percentage of carbon dioxide produced in the desiccator in three hours under ordinary pressure was determined. The air in the desiccator was then changed and fresh air was admitted. The desiccator was then evacuated up to a vacuum of 240 mm. of mercury (approximately one third atmosphere) and the respiration of the sample was allowed to proceed under the reduced pressure. At the end of three hours, the vacuum was released by allowing fresh air into the desiccator. The percentage of carbon dioxide produced in the desiccator was determined. Similarly, the percentage of carbon dioxide produced by the same

sample in three hours under a vacuum of 480 mm. of mercury (approximately two thirds atmosphere) was determined. As all the three determinations were made in the same desiccator and for an equal period, the percentage of carbon dioxide produced in the desiccator indicated the intensity of respiration of the fruit. The results are given in Table VI.

TABLE VI

Respiratory activity under partial vacuum at 68°F.

	Percentage of carbon dioxide produced			
	Green fruit		Ripe fruit	
	Sample I	Sample II	Sample I	Sample II
Atmospheric pressure (710 mm.)	9·4	12·8	4·0	4·2
240 mm. vacuum . . .	10·8	13·2	..	..
480 mm. vacuum . . .	11·0	11·0	5·2	4·5

The results in Table VI show that respiratory activity did not decline under reduced pressure but on the other hand, the values obtained indicate that there was probably some increase. If it is assumed that the browning of the yellow skin of ripe fruit when stored at 52°F. is due to the breaking of the cells in the skin and the subsequent oxidation of the cell contents by exposure to the atmospheric oxygen, the prevention of the development of brown colour could only be explained by the reduced quantity of oxygen under conditions of partial vacuum.

EFFECT OF CARBON DIOXIDE CONCENTRATION ON RESPIRATION

In the experiments described above, the period of the respiration experiment was so arranged that the concentration of the accumulated carbon dioxide produced in respiration did not normally exceed 10 per cent. High concentrations of carbon dioxide are generally supposed to have a depressing effect on respiratory activity. The effect of different concentrations of carbon dioxide on the rate of respiration of the Alphonso mango was studied. Two samples of green fruit were used. The respiration was allowed to continue at 68°F. for 24 hours when a high concentration of carbon dioxide was obtained. At intervals during the experimental period the percentages of carbon dioxide accumulated in the respiration jars were determined. For each determination 100 c.c. of the gas were obtained by displacement by an equal quantity of water. A correction for the amount of carbon dioxide absorbed by water remaining in the respiration jar was made in calculating the total quantity of carbon dioxide produced by the fruit. The data obtained are given in Table VII.

The total volume of carbon dioxide produced at intervals during the experimental period (Table VIII) has been represented graphically in fig. 2.

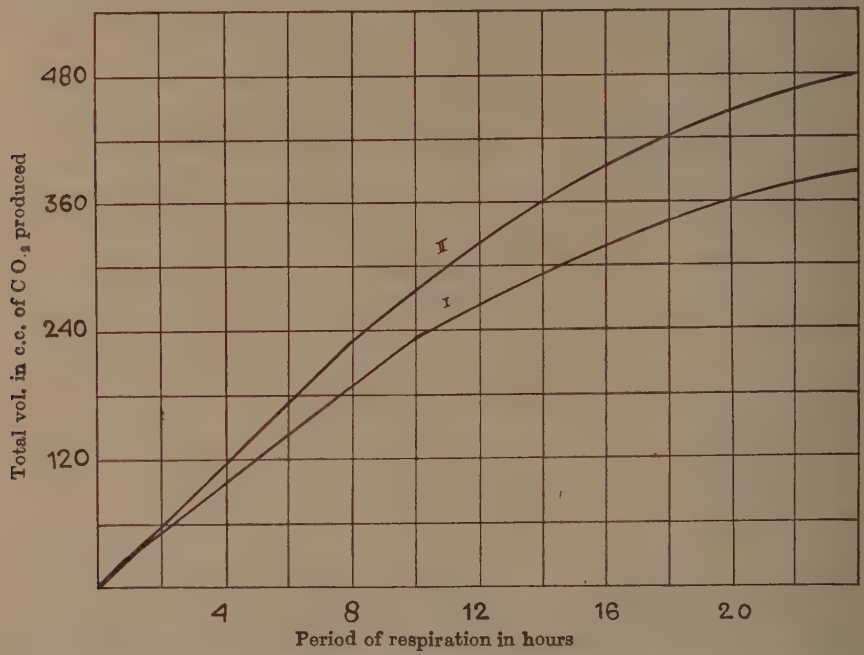


FIG. 2. The effect of increased concentrations of carbon dioxide on the respiratory activity of Alphonso mango

It will be observed from the figure that in sample I the rate of production of carbon dioxide was uniform up to 10 hours when the rate commenced to decline. The concentration of carbon dioxide at this point, i.e. after 10 hours, would have been approximately 11 per cent. In sample II, the rate was uniform up to eight hours when the concentration of carbon dioxide had reached 11 per cent. It can, therefore, be concluded that the accumulation of carbon dioxide in the respiration jar up to a concentration of about 11 per cent did not produce any depressing effect on the respiratory activity. In these experiments, the percentage of oxygen in the closed respiration jar was reduced corresponding to the increase in the carbon dioxide concentration, the respiratory quotient being about unity. The reduction in the percentage of oxygen from 21 per cent to 10 per cent did not, therefore, have any retarding action on respiratory activity. Indications of anaerobic respiration were not obtained even after an accumulation of 12·8 per cent of carbon dioxide in sample I and 15·4 per cent in sample II, i.e. after a reduction of oxygen percentages to 8·2 and 6·6 respectively. Definite anaerobic respiration occurred after 24 hours, i.e. after an accumulation of 19·2 per cent of carbon dioxide in sample I, and more than 21 per cent in sample II. Singh, Sheshagiri and Gupta [1937, 2] have noticed that an oxygen mixture of 9·2 per cent was the critical concentration below which there was probably competition between aerobiosis and anaerobiosis, the respiratory quotient at this critical point being 0·85.

Respiration under anaerobic condition

The respiration was allowed to continue at 68°F. until the concentration of carbon dioxide produced in the respiration jar was considerably more than 21 per cent, i.e. the percentage of oxygen in the air. It was observed that when all the oxygen was consumed and anaerobic respiration commenced, the manometer showed an increase of pressure. In a few samples, specially when the concentration of carbon dioxide produced was more than 30 per cent, the increase in pressure was not proportional to the anaerobic production of carbon dioxide but was much less. On calculation, it was found that in these cases there was a considerable decrease in the amounts of nitrogen (calculated by difference) in the respiration jar. The data appeared interesting and are recorded in Table VIII. No conclusion could be proposed as a result of these few stray observations until further corroborative data are obtained.

Table VII

The effect of the concentration of carbon dioxide on the rate of respiration

Period of respiration in hours	Sample I				Sample II			
	Percentage of CO_2 in the respiration jar	Total volume of CO_2 produced c.c.	Volume of CO_2 produced in the interval c.c.	Volume of oxygen consumed in the interval c.c.	Percentage of CO_2 in the respiration jar	Total volume of CO_2 produced c.c.	Volume of CO_2 produced in the interval c.c.	Volume of oxygen consumed in the interval c.c.
1	1·2	23	23	29	1·4	28	28	18
2	2·4	47	24	25	2·6	53	25	30
4	4·8	95	48	48	5·4	110	57	58
8	9·3	185	90	84	11·0	225	115	105
12	12·8	258	73	71	15·4	320	95	86
24	19·2	389	131	71	23·0	480	160	48

Summary

1. In previous investigations on the cold storage of mango, it was found that the Alphonso mango was the best keeper in cold storage. The respiratory activity of this variety of fruit under different storage conditions has been studied.

2. The respiratory activity of green fruit ('B' stage of maturity) increased on keeping at 68°F., reached a peak value (climacteric) and then decreased during ripening. The 'B' stage of maturity—the condition found most suitable for cold storage—occurred in the pre-climacteric phase of the life-cycle of the fruit.

3. During ripening, the decline in the rate of respiration was accompanied by an increase in the amount of total sugars and a loss in acidity. It was inferred, therefore, that the rate of respiration was not influenced by the increase in the concentration of sugars and that it decreased due to the depletion of acids present in the fruit. The values of the respiratory quotient indicate that the acids formed a part of the respiration substrate.

TABLE VIII

Respiration under anaerobic condition

Period of respiration in hours	Volume of fruits c.c.	Original volume of air: oxygen (20·7 per cent) / nitrogen in the jar c.c.	Increase in pressure mm. of mercury	Volume of air in the jar at atmospheric pressure (710 mm.) c.c.	Percentage of CO_2 in the jar	Percentage of oxygen in the jar	Volume of CO_2 in the jar at 68°F. and 710 mm. pressure c.c.	Volume of oxygen in the jar at 68°F. and 710 mm. pressure c.c.	Volume of nitrogen in the jar at 68°F. and 710 mm. pressure (by difference) c.c.
43	605	2095 / 433 / 1662	64	2283	27·6	0·5	630	11	1642
26	578	2122 / 439 / 1683	93	2400	29·8	0·4	715	10	1675
43	539	2161 / 447 / 1714	104	2477	30·9	0·6	796	15	1666
26	498	2162 / 454 / 1708	103	2476	30·2	0·4	748	10	1718
24	452	2248 / 476 / 1772	63	2446	33·0	0·4	807	10	1629
24	498	2202 / 467 / 1735	66	2406	34·6	1·2	833	29	1544
26	664	1976 / 411 / 1565	15	2017	40·0	0·5	807	10	1210
48	539	2161 / 447 / 1714	54	2325	44·0	0·4	1023	9	1293
44	525	2175 / 461 / 1714	64	2371	46·0	2·0	1091	47	1233
45	500	2200 / 455 / 1745	73	2426	50·0	0·2	1213	5	1208

4. The respiratory quotient of fresh fruit of 'B' stage of maturity was less than unity and the manometer attached to the respiration jar showed a decrease in pressure in the jar. The depression in pressure could be employed as a test for the freshness of the fruit.

5. The rate of respiration decreased with temperature. There was, however, a marked decrease between 52°F. and 48°F.

6. The rate of respiration declined in the first fortnight of storage at 48°F., but afterwards, it remained steady up to the end of the storage period (six weeks). The rate of respiration of fruit removed to 68°F. after storage at 48°F. did not decrease during ripening.

7. Chilling injury did not appear to have any marked effect on the rate of respiration.

8. The determinations of the respiratory activity of fruit kept under partial vacuum showed that the rate of respiration was not affected by the reduced pressure.

9. It was observed that the accumulation of carbon dioxide in the respiration jar up to a concentration of 11 per cent did not produce any depressing effect on the respiratory activity.

10. In a few samples, the increase in pressure in the respiration jar was not proportional to the anaerobic production of carbon dioxide. It was observed that there was a considerable decrease in the quantity of nitrogen in the respiration jar specially when the concentration of carbon dioxide produced was more than 30 per cent. No conclusion could be proposed as a result of these observations until further corroborative data are obtained.

Acknowledgements

The authors are greatly indebted to the Imperial Council of Agricultural Research for subsidizing the Cold Storage Research Scheme, which is under the supervision of Dr. G. S. Cheema, D.Sc., I.A.S., Horticulturist to Government, Bombay Province, Poona. These respiration studies of the Alphonso mango formed a part of the cold storage investigations on the mango in progress under that scheme.

The authors wish to acknowledge the help of Messrs. R. K. Lavlekar and S. W. Rane in the experimental work.

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STUDIES ON THE FORMATION OF JELLIES FROM SOME INDIAN FRUITS

BY

BIRENDRA NARAIN SINGH

AND

SIKHIBHUSHAN DUTT

Chemical Laboratory, University of Allahabad, Allahabad

(Received for publication on 22 May 1941)

INDIA possesses such wide range of climate and soil that there is no fruit of the temperate, the subtropical and the tropical regions that cannot be grown here, and many grow so abundantly that huge quantities go to waste every year. Yet India gets its supplies of fresh and preserved fruits in large quantities every year from foreign countries. The problem of utilization of this abundant supply of fruit, which is of such vital importance to the development of the fruit industry in this country, has unfortunately not received any great attention from chemists or industrialists. Although some systematic research on the preservation of fruits and vegetables in India has been in progress in the Fruit Products Laboratories, Lyallpur, yet the field of research in this line is so vast and the literature on Indian fruits so scanty, that the problem of making jellies from some Indian fruits was considered of sufficient importance by the present authors so as to deserve a careful and systematic study.

In the present investigation the optimum conditions of jelly formation from some Indian fruits have been made the subject of scientific study, involving a good deal of physico-chemical analyses of the fruits and the jellies with special reference to the relationships of sugar, acid and pectin most desirable for the formation of a highly statisfactory jelly. The industrial aspect of the problem has also received careful consideration and it is hoped that the data collected will serve a useful purpose.

According to Goldthwaite [1918], a fruit jelly is a beautiful coloured and transparent product of fruit juices, which is neither syrupy, gummy, sticky or tough, nor brittle, and it cuts easily with a spoon, leaving sparkling characteristic faces the angles of which retain their shape. Perhaps no better definition of the fruit jelly can be given.

The chemistry of jelly formation is chiefly concerned with pectin, acid and sugar concentrations. These three constituents form a rather definite equilibrium when jelly formation occurs. Time of cooking, temperature, salt concentration and the like are also important considerations, but their importance rests more particularly upon the manner in which they affect the pectin, acid and sugar constituents.

Pectin

According to Meyers and Baker [1934] pectin in the unhydrolysed condition is mono-arabino-mono-galacto-diacetylheptamethoxyl-octagalacturonic

acid. Nucleus of the pectin molecule is octa-galacturonic acid most likely formed by the union of two molecules of tetra-galacturonic acid with the elimination of one molecule of water. Tetra acid is most likely formed into a ring compound by the combination of four molecules of galacturonic acid with the elimination of four molecules of water. Seven of the eight carbcxyl groups of the octa-galacturonic acid are methylated, and the other one is free. On this basis the empirical formula for pectin would be $C_{70}H_{98}O_{58}$ with a molecular weight of 1,866,784.

Further, Baker and Goodwin [1939] while reviewing the work of various authors have stated that ' Present opinion definitely favours the assumption that pectin is a chain compound composed of galacturonic acid groups.....In this chain compound the carboxyl groups are 75 per cent methylated (11·92 per cent CH_3O) and the position of the free carboxyl is arbitrary.'

In rotten fruit proto-pectin disappears, pectin is present to some extent, while pectic acid and methyl alcohol exist in excess. The jellying properties of these are distinctive. Proto-pectin does not form a jelly when cooked with sugar and pectin-free juice. Pectin is the substance to which the jellying of the fruit juice is due. Pectic acid also does not form any jelly, like pectose. Fallenberg [1918] bases the action of various pectic bodies on the methoxy groups which they contain. Sucharipa [1923] carrying out experiments with increased temperature and pressure, obtained pectins of decreased methoxy contents, and the jellying power has been stated to be a direct function of their methoxy contents. This statement is, however, contradicted by Meyers and Baker [1934] who state that 'Contrary to general belief the methoxy content of pectin is not a measure of their jellying power'.

Estimation of pectin by precipitation with alcohol is not reliable. The method of Emmett and Carre [1926] as modified by Nanji and Norman [1928] has been used for the quantitative estimation of pectic substances in the present investigation. The results have been expressed as yield of calcium pectate per 100 gm. of the dried powder of the fruits.

Acid

Cruess and McNair [1916] determined that the optimum acidity of good jellying fruits lay within 0·5—1·5 per cent of acid as citric acid. Campbell [1920] states that 0·3 per cent of acid as sulphuric acid is necessary to produce a good jelly. The presence of 0·86 per cent of sulphuric acid is, in the opinion of Goldthwaite [1918], necessary to produce good jellies from sour apples. Lal Singh [1922] is stated to have made jellies from a mixture of pectin, acid, sugar and water even when it contained as low as 0·05 per cent of citric acid. Tarr [1923] determined a direct relationship with hydrogen ion concentration and formation of jelly, the minimum *p*H being 3·46—a value which is independent of the nature of the acid used. The hypothesis of pectin-acid compound, or the stoichiometric relation between them, finds no substantiation in the works of Gene Spencer [1930] who states that pectin increases the hydrogen ion concentration and does not act as a buffer tending to suppress it, as expressed by Tarr [1923].

Sugar

Although jellies have been made without any addition of sugar, in general, sugar in the process of jelly making determined the texture, appearance

and flavour of the jelly, as well as the yield of the final product. Although sugar can be varied to a great extent for the production of a jelly, but a rather definite proportion of the substance is needed for the formation of a good jelly, and the percentage of sugar existing in the finished product is reasonably constant.

Materials and methods of analyses

Materials

A brief account of the various materials used in this investigation is given below :—

Feronia elephantum.—Natural Order Rutaceae. Known as elephant or wood apple in English and *Kaitha* in Hindi, is the fruit of a large deciduous tree grown throughout India in great abundance. Its profusion coupled with the great cheapness of the fruit has lowered it in the estimation of the people to such an extent that it is hardly ever eaten by any one. It has, however, been found by the present investigators to be an excellent jelly forming material, being very rich both in acid and pectin. Estimations made at different stages of ripeness of the fruit show that the fruit is most suitable for jelly formation when just ripe.

Psidium guava.—Natural Order Hyrtaceae. Known as guava in English and *Amrud* in Hindi, is a fruit of a low tree, cultivated almost all over India. It is quite rich in pectin, but is incapable of forming jellies as the fruit is deficient in acid. The various varieties grown in India have been classified as Narma, Safeda, Chittidar, Kakra, Karela, Hafni and Seedless, all being well known. Estimations made with each of the above varieties show that Safeda, Chittidar and Kakra varieties are most suitable for jelly making, being richer in pectin than other varieties in their ' just ripe ' and ' fully ripe ' stages.

Carissa carandas.—Natural Order Apocynaceae. Known as *karounda* in Hindi, it is the fruit of a thorny shrub grown throughout India. The fruit is a berry, ½—1 in. long, smooth and reddish purple when ripe. On examination, it has been found to be very rich in acid and pectin, and as such it yields excellent jellies.

Hibiscus sabdariffa.—Natural Order Malvaceae. Known as roselle or red sorrel in English and *patua* in Hindi, is a low shrub that is largely cultivated in India for its pleasant acidulous calyxes during the hot seasons. The red calyx which encloses the entire fruit is very rich in acid and pectin and as such is an excellent source of fruit jellies.

Citrus nobilis Lour.—Natural Order Rutaceae. Known as mandarin in English and *santra* in Hindi, it is the fruit of an evergreen tree of moderate size, grown chiefly in warm and moist regions of India. The fruit has been found to be fairly rich in pectin, but the acid present is not sufficient to form a a good jelly.

Citrus aurantifolia Swingle.—Natural Order Rutaceae. Known as sour lime in English and *nimboo* in Hindi, it is the acid fruit of a low tree which is widely cultivated in many parts of India. It contains 7—8 per cent of citric acid and is very rich in pectin, and forms excellent jellies, which are unfortunately too sour. In view of the fact that the fruit is very rich in acid and pectin its juice is often admixed with other fruit juices which are deficient in them so as to form good mixed jellies.

Zizyphus jujuba.—Natural Order Rhamneae. Known as jujube fruit in English and *ber* in Hindi, it is the fruit of a medium sized tree often found wild and cultivated in many parts of India. The fruit is a drupe, orange or red when ripe, the stone forming 17-20 per cent of the whole fruit. The fruit contains a fair proportion of pectin, but is deficient in acid and only forms good jellies on supplying the extra acid needed.

Musa paradisca.—Natural Order Musaceae. Known as banana in English and *kela* in Hindi, it is the fruit of a tall herb cultivated throughout India for its nutritious and delicious fruit. The peel forms 10-12 per cent of the fruit which is yellow when ripe and green when unripe. The fruit is fairly rich in pectin, but deficient in acid and so does not form any jelly unless extra acid is added.

Aegle marmelos.—Natural Order Rutaceae. Known as *bel* in Hindi, it is the large fruit of a deciduous tree which grows throughout tropical India mainly in the cultivated form. The pulp of the fruit is orange-yellow in colour, many seeded with a transparent and exceedingly sticky gum. It is quite rich in pectin, but very deficient in acid and on that account does not form any jelly unless extra acid is added.

Physalis peruviana.—Natural Order Solanaceae. Known as cape gooseberry in English and *makoi* in Hindi, it is the fruit of a low shrub which is cultivated in many parts of India. The fruit is a berry, which when ripe is of a bright yellow colour enclosed within the membranous calyx which forms 6 to 8 per cent of the whole fruit.

Methods

The various methods employed for different physico-chemical examinations and in the determination of certain jelly properties are given below :—

1. *Moisture.*—A known weight of the freshly cut fruit was dried to a constant weight in the steam oven, and from this the moisture percentage was calculated.

2. *Pectic substances.*—The quantitative estimation of pectin was made by the method of Nanji and Norman [1928] with certain variations in time and temperature of extraction as given below :—

All enzymes were first destroyed by placing the fresh fruit in thin layers in the steam oven. Thin slices were then cut and dried in enamelled dishes at 92° C. and then ground to a fine uniform powder so as to effect the complete extraction of the pectic substances. As many of the powders were hygroscopic and absorbed moisture from the air rapidly, they were all kept inside a large desiccator.

Equal amounts of each sample (varying between 3 and 5 gm.) were separately extracted with (A) pure water, which removes free pectin, (B) 0·5 per cent oxalic acid solution, which removes pectin and pectose and (C) 0·5 per cent of ammonium oxalate solution, which removes free pectin, pectose and free and combined pectic acid. Extraction was complete after 18 hours' heating at 87°C. The extracts were filtered through fluted filter paper, the residue washed with the same solvent and the total extract and washings made up to 250 c.c., 100 c.c. of each extract were concentrated to 1/3rd the original volume, the extract with oxalic acid solution being neutralized before concentration to avoid hydrolysis. Pectic substances in these extracts, cooled to

room temperature were precipitated as usual with acidified alcohol. The alcoholic precipitate after washing free from oxalate was disssolved in water containing a few drops of ammonia, and the pectin estimated in the solution obtained by the usual calcium pectate method. Quantities of pectin, pectose and free and combined pectic acid were calculated from the calcium pectate figures of the three extracts (Appendix, Table I), as under : A=pectin ; B—A=pectose ; C—B=pectic acid, free and combined. At least two and in some cases six to seven samples of fruits were thus estimated for. Total pectic substances (C) has been calculated on 100 gm. of fresh materials, under pectins in Table II given in the appendix. The estimation of pectin in the fruit juice for jelly making has been done as in (A).

3. *Sugars.*—They were estimated as total sugars and reducing sugars expressed in terms of glucose, by the usual Fehling's method.

4. *Acids.*—The total acids have been determined by titration with standard caustic soda solution, expressed in terms of citric acid.

5. *Method of jelly making.*—The best stages of the fruits for jelly making purposes are the just ripe and the well ripe varieties. Their main constituent, responsible for the jelly forming property, is free pectin. It being soluble in water is extracted easily. The pectose present is also hydrolysed to free pectin by the hot extraction usually employed for jelly formation from fruit juices. The clear juice obtained by filtering through a piece of flannel was estimated for its acid and pectin content. Measured volumes of juices were boiled with known amounts of cane sugar in weighed beakers and concentrated to the jelly forming point. The mixture darkened in colour and tended to boil over which was carefully avoided by regulating the flame.

Sheeting test.—A juice rich in acid and pectin begins to jell at 105°C. It has to be concentrated further when the acid and the pectin contents are poor. Another test consisted in taking the hot syrup on the handle of a spatula and allowing it to fall from above. A syrup which would definitely gel on cooling, showed a tendency to break in a ' sheet ' form the edges of the spatula instead of falling in drops. This test recommended by Tarr and Baker [1924] has been used in this investigation. The hot syrup was allowed to cool for 24 hours. The greater the amount of pectin and acid in the finished product, the quicker was the setting point of the jelly.

6. *Determination of jelly strength.*—The thick syrup obtained after testing the jellying point or temperature, was cooled down to 80°C. and then transferred to a weighed Ostwald Viscometer of 3 mm. bore, fitted with a rubber tube and pinchcock arrangement. After setting of the jelly, the rubber tubing was attached to the manometer, which in turn was attached to the suction pump recording an uniform pressure of 20 cm. of mercury. The jelly moved forward steadily and the time it took to go through a particular length of the tube was noted by a stop watch. The times required by different jellies under uniform conditions of experiment are expressed in seconds under the column 'jelly strength'. The ideal jelly required 83 -85 seconds, and 9 seconds indicated a bare gel formation. This method of determination of the relative strengths of jellies was suitable for a fair range of stiffnesses of these materials, but was unsuitable for very tough or sticky jellies.

7. *Determination of sineresis temperature.*—About 5 gm. of the jellies were taken in a test tube and kept in a beaker half filled with water. The

temperature of the water was raised slowly by a regulated flame, and at the first sign of the formation of drops of clear fluid in the jelly, the temperature was noted. The various temperatures obtained have been tabulated in the column of ' sineresis temperature'. It was found to be 60°C. in the case of good jellies, showing thereby their stability even through the hot months of the year.

Sugar pectin and acid relationship in the formation of jellies

1. *Effect of sugar.*—With a measured volume of fruit juice, jellies were prepared with increasing amounts of pure crystalline cane sugar. The jelly strength and sineresis temperature were determined. The experimental results obtained with wood apple juice containing 0·238 gm. citric acid and 0·07 gm. pectin (as calcium pectate) are expressed in Table III. Although the quantities of sugar employed varied over a considerable range, the percentage of sugar in the finished jellies was constant within a reasonable limit of error. Jellies made with guava juice containing 0·076 gm. pectin, 0·418 gm. acid and increasing amounts of sugar gave the results expressed in Table IV. Greater yield with decreased toughness in the jellies resulted with increase in the amounts of sugar. The exact amount of sugar necessary for the production of an ideal jelly was also determined. The amounts of acid, pectin and sugar in the finished jelly have also been calculated, indicating the percentage composition of various jellies. Effect of increasing amounts of sugar on roselle juice containing 0·235 gm. acid and 0·12 gm. pectin has also been tried. Table V shows the results. Addition of increased amounts of sugar was continued until the jelly formation was prevented. The failure of jelly formation with a fruit juice even though it may be quite rich in acid and pectin is due to the addition of too much of sugar, which is beyond the holding capacity of the pectin. The importance of such experiments can thus be easily realized, since they indicate the right concentration of sugar for the production of an ideal jelly. Effect of increasing amounts of sugar was also tried with juices obtained from several other Indian fruits like *karounda*, jujube, banana, orange, lemon, *bel* and cape gooseberry, establishing the optimum conditions of their jelly formation.

2. *Effect of acids.*—Normal solutions of citric, tartaric and hydrochloric acids were added in increasing amounts to the same quantity of wood apple juice in different beakers. The percentage of pectin and sugar was kept constant at 0·357 and 59·5 per cent respectively, by adding equal amounts of sugar and boiling the solutions off to a constant weight. Table VI shows the jellies of different strengths thus obtained. It was observed that up to a certain limit, increase of acidity increases the jelly strength. With a further increase of acid, a decrease in the jelly strength resulted, and excess of it produced a syrup which completely failed to jell.

The explanation of the above behaviour is fully borne out by Tarr's [1923] view. He conceives of fruit jellies as possessing theoretically the usual cellulose structure of colloids. The colloid exists in the cell walls in a viscous form and is thereby capable of holding the liquid contents of the jelly up to a certain limit of acidity. The cell walls become firmer and firmer as the acidity is increased, resulting in jellies of greater strength, and also increasing

the holding capacity of the cell wall. On increase of acidity beyond a certain limit, the pectin becomes precipitated in the cell wall in a more or less granular form thereby decreasing the liquid retaining properties of the cell walls to a very considerable extent. Under such conditions only syrupy jellies of low strength are obtained. On increasing the acidity still further, even the cell walls become granular and their liquid retaining properties cease altogether. Consequently, no jellies are formed. As to the effect of acids, citric acid produces lesser increase in jelly strength than the same amount of tartaric acid, while only minute quantities of hydrochloric acid were required to produce the same jelly strength.

Similar sets of experiments were performed with guava juice which is quite deficient in acid. Sugar was kept constant at 59·5 per cent and pectin at 0·286 per cent in the finished jelly. Table VII shows the effect. As is clear from the table, at least 7 c.c. of citric acid and 5 c.c. of tartaric acid are required for a distinct jelly, the concentrations in the finished products being 1·46 and 1·02 per cent respectively. Lesser amounts produced only thick syrupy masses. As regards hydrochloric acid, even 0·5 c.c. yielded a product of 67 jelly strength (Table VII), whereas further increase in its amount, in contrast to citric and tartaric acids, tended to decrease the jelly strength with increasing production of bitter taste. Tartaric acid besides being more effective than citric acid, formed jellies with better flavour, texture and colour.

As has been observed above that the increase of acidity increases the jelly strength, with increased acid a decrease in the amount of sugar can be effected to get a jelly of the same strength, and thus 20 per cent sugar has been saved by Lal Singh [1922]. The sugar holding capacity of the pectin also increases with increase of acid, thus resulting in a greater yield of the jelly. Similar experiments were conducted by Tarr [1923] with increased hydrogen ion concentration. The strength of the jelly prepared from a fruit juice was determined. A known amount of tartaric acid was then added to equal quantities of the same juice and the amount of sugar was varied to get a jelly of that very strength. More of acid was added and the corresponding increase in the amount of sugar was determined, till it was found that further increase in acidity did not increase the amount of sugar and if done so, a jelly of lower strength resulted. With 0·095 gm. of pectin in *karounda* juice (Table VIII), 40 gm. of sugar were required at 0·51 per cent acid. On increasing the acidity to 1·6 per cent, the increase in the amount of sugar was found to be 4 gm. Expressing the result with 1 gm. of pectin (as calcium pectate), it could hold 42 gm. of sugar more at 1·6 per cent acidity than what it could at the original acidity. Similarly, guava juice containing 0·134 gm. pectin could hold 30 gm. of sugar (Table IX), but at 2·65 per cent acidity the same amount of pectin could hold 38 gm. of sugar. With 1 gm. of guava pectin, as much as 60 gm. more of sugar can be held at the increased acidity. Similar results were obtained with juices of wood apple and roselle, holding about 70 gm. of sugar more at an acidity of 2·48 per cent. As the tables show, increase of acid increases the percentage of sugar in the finished product. This is due to the greater inversion of the cane sugar, thereby increasing its solubility. Thus more sugar was required to get an approximately saturated solution of it, at which the precipitation of the pectin in the form of jelly occurs. The syrup had to be concentrated further before a tendency to jell was observed.

3. *Effect of pectin.*—Increase of pectin increases the jelly strength and decreases the percentage of sugar at which it begins to jell. Pectin extracted in the usual manner was dissolved in warm water and a concentrated solution obtained. The calcium pectate yield of 10 c.c. of this solution was found out and the solution was used as the source of pectin. The strength of a jelly prepared from a fruit juice was determined and the yield noted. A known volume of the pectin solution was then added to equal quantities of the same juice and the amount of sugar was varied in order to get a jelly of that particular strength. The yield of the jelly was maintained to keep the percentage of the acid constant. Increased volumes of the pectin solution were added and the corresponding decrease in the amount of sugar was determined. Results obtained with wood apple juice are shown in Table X. 10 c.c. of the pectin solution used had a calcium pectate yield of 0·32 gm. 30 gm. of sugar was added to the juice containing 0·07 gm. pectin, when the finished jelly contained 66·6 per cent sugar and 0·16 per cent pectin. On increasing the pectin to 1·57 per cent, the amount of sugar required was only 23 gm. and the finished product contained 51·1 per cent of sugar. On increasing the pectin to 1·93 per cent, the upper layer of the jelly became stiffer and rather bitter showing excess of pectin. It thus appeared that within certain limits, the higher the percentage of pectin in the juice, the lower is the percentage of sugar required to form the jelly. This was well marked till there was about 1·5 per cent of pectin in the jelly, after which further increase of pectin did not decrease the amount of sugar to any great extent. Similar results were obtained from pectin derived from roselle calyx, 10 c.c. of its prepared solution being equivalent to 0·48 gm. calcium pectate . 40 gm. of sugar were needed to produce a jelly with 0·24 per cent of this pectin and the finished product contained 67·8 per cent of sugar. On increasing the pectin to 1·45 per cent, only 53·4 per cent sugar in the finished product was obtained with the same jelly strength. Thus about 15 per cent of the sugar can be saved by increasing the pectin content to 1·5 per cent in the finished jelly (Table XI).

It has been observed in both the above cases that the presence of less than 1·5 per cent of pectin in the jelly showed no distinct taste of the pectin and the jellies were quite normal. When the percentage of pectin was raised further than this point, on the upper part of the jelly, a crust-like formation appeared which was stiffer and less sweet than the lower portion. At about 2 per cent pectin and above, the upper layer besides being more stiff was distinctly bitter in taste, showing that pectin beyond 1·5 per cent (as calcium pectate) remains inactive and undissolved. Lal Singh [1922] observed no change till approximately 2·5 per cent pectin. after which the above abnormality was observed by him. This might be due to the fact that his pectin estimations are expressed in terms of alcohol precipitates which contain many other impurities besides pure pectin.

Experiments with different kinds of fruits

Feronia elephantum.—The fruit is an excellent material for jelly making. The inner white pulp attached to the hard rind of the wood apple is also fairly rich in pectin, which can be commercially extracted from it. The method consisted in treating the slices with water or 0·5 per cent tartaric acid solution

and heating for 1½ hours at 90-92°C. The liquid extract was filtered and the residual pulp extracted twice more in the same way. The combined extracts were concentrated and the pectin precipitated with acidified alcohol. It was filtered, washed, dissolved in hot water and reprecipitated with alcohol. The second precipitate was filtered and thoroughly washed. It can be dried by heating under reduced pressure or can be dissolved again in water forming a concentrated pectin solution and stored in bottles for use with fruit juices which are deficient in it.

Psidium guava.—The extraction of fruit juice for jelly making is best effected with 0·5 per cent tartaric acid. In actual experiments extraction done with water yielded 0·72 per cent of pectin, whereas with taratric acid, 1·04 per cent of pectin was extracted, clearly showing its favourable effect. In another experiment jelly was made from guava juice and 0·5 per cent acid, but without any addition of sugar. The resulting product was found to be a very tough dark red jelly. A more interesting experiment made with guava jelly is described below :—

Equal amounts of juice and sugar were boiled off until the temperature reached 105° C. The product being deficient in acid, formed a thick syrup, but no jelly. To each of the beakers normal tartaric acid solution in lots of 5, 10, 15 and 20 c.c. were added in cold. Observations made after 24 hours showed that the beaker containing 20 c.c. of acid had jelled to a fair degree, others to a less extent. After 48 hours fairly stiff jellies were formed in beakers containing 20 and 15 c.c. of acid. That containing 10 c.c. of acid formed a bare gel, whereas no jelly was formed with 5 c.c. of acid. Formation of jellies under such conditions clearly brings out the idea expressed by Tarr and Baker [1924] that precipitation of pectin in the form of jelly occurs in a saturated or nearly saturated solution of sugar, which also seems to be controlled by the active acidity. The greater the amount of acid, the quicker was the precipitation of the pectin in the form of jelly, as observed above. Jellies made at room temperature by Cruess [1922] and Sucharipa [1923] also prove the same interesting fact.

Carissa caraundas.—The fruit being very rich in pectin and acid forms very good jellies. An ideal jelly resulted with juice containing 0·316 gm. acid, 0·072 gm. pectin and 17·5 gm. sugar made in the usual way.

Hibiscus sabdariffa.—Jellies were made out of all the three parts of the fruit. The entire fruit resulted in a jelly which was a little slimy and loose. The fruit pods alone formed a sticky syrup and on addition of acid a bare gel was formed which was also sticky. Jellies made with calyx alone formed very nice jellies of a bright red colour. Analyses of the three parts also showed that the maximum amount of pectin and acid were in the calyx and the least in the fruit pod.

Citrus nobilis Lour.—A sticky mass resulted with the fruit juice and sugar. With gradual addition of acid, jellies of increasing strength were obtained till at 1·7 per cent acid, a fairly stiff jelly resulted, having a good orange flavour.

Citrus aurantifolia Swingle.—Jellies made with the extracted juice and sugar were quite stiff, but the product was too sour and had a slightly bitter taste also The fruit being very rich in acid and pectin is often mixed with other fruit juices deficient in them.

Zizyphus jujuba.—Boiling the fruit juice with sugar resulted in a sticky mass and no jelly. Addition of acid formed the jelly. With low concentration of acid, the jellies were sticky, but the stickness disappeared with increase of acid.

Musa paradisca.—The milky juice extracted from the fruit formed jellies only on the addition of acid and a concentration of 1·7 per cent of acid in the finished product resulted in a fairly stiff jelly. The banana flavour which disappears during boiling is retained by the addition of a few c.c. of concentrated juice at the jellying point.

Aegle marmelos.—The fruit although quite rich in pectin, did not form jelly with increased acid even to the extent of about 2 per cent in the finished product. This is due to the fact that the juice having a peculiar odour, contains excess of a gummy principle which checks the jelly formation. After three days the syrup formed a bare gel which too was very sticky.

Physalis peruviana.—A concentrated extract of the fruit juice with sugar and 1·5 per cent acid resulted in a tender jelly. Efforts to make stiffer jellies failed, as the fruit is poor in both acid and pectin.

Mixed jellies.—Jellies of mixed aroma and flavour resulted by mixing one fruit juice rich in pectin and acid with another having a good flavour. Experiments performed in this way show that four parts of guava and two parts of lemon, five parts of wood apple and one part of lemon, four parts of orange and two parts of wood apple produce ideal jellies, having mixed aroma and flavour.

Summary

1. A quantitative estimation of acids, pectin, sugars and moisture of some Indian fruits has been made, showing their suitability for jelly making.

2. Quantitative estimations of the three pectic substances, namely free pectin, pectose and free and combined pectic acid in the above fruits were made by Nanji and Norman's [1928] method of differential extraction, with certain variations, which greatly diminished the period taken in the estimation.

3. Optimum conditions of formation of jellies from some Indian fruits have been found out, the jelly strength and the temperature of sineresis determined.

4. Effect of mineral and organic acids on the jelly formation has been studied. Increase of acidity increases the jelly strength to a certain limit, after which it is decreased. The sugar holding capacity of pectin increases considerably by increase of acid, and tartaric acid has been found to be more effective than citric acid in this respect, besides producing a better flavour.

5. Increase of pectin decreases the percentage of sugar at which the syrup begins to jell, but increase of pectin beyond 1·5 per cent in the finished product remains inactive and undissolved, resulting in abnormal jellies with somewhat bitter flavour.

6. A definite amount of sugar is required for a particular juice to result in an ideal jelly depending upon its acid and pectin contents, and a definite equilibrium exists between sugar, acid, pectin and water when jelly formation occurs.

7. The percentage composition of ideal jellies lies between 65 and 70 per cent total solids, containing 0·75—1·75 per cent acid as citric acid, 0·25—0·5 per cent of pectin as calcium pectate and 61—65 per cent of sugar.

8. Employing total acidity as the criterion of the acid content of a frut jelly, substantiation of the theory put forward by Tarr and Baker [1924] has been made, which states that a fruit jelly formation appears to be a precipitation of pectin in approximately saturated solution of sugar, and this seems to be controlled by active acidity.

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APPENDIX

Table I

Estimation of pectins

(Percentage of pectic substances in the dried powder of the fruits)

Fruit	A (water)	B (oxalic (acid)	C (Ammo-nium oxalate)	B-A	C-B
Wood apple—					
A. Pulp	10·54	12·95	14·05	2·41	1·1
B. White peel	9·75	14·15	19·2	4·4	5·05
Guava	4·26	6·08	6·89	1·82	0·81
Karaunda	5·41	9·16	11·24	3·75	2·08
Roselle—					
A. Calyx	17·52	23·25	27·1	5·73	4·05
B. Entire fruit	9·52	13·15	14·35	3·63	1·2
C. Fruit	3·12	4·05	4·31	0·93	0·26
Orange	8·85	10·7	11·85	1·85	1·15
Lemon	13·65	17·14	18·55	3·49	1·41
Jujube (*ber*)	3·9	6·52	7·28	2·62	0·76
Banana	2·52	3·2	4·53	0·68	1·33
Bel	2·75	4·6	6·57	1·85	1·97
Cape gooseberry	2·05	4·25	4·65	2·2	0·4

Table II

Analyses of fruits

(Figures indicate percentage of various components in fresh fruits)

Fruit	Acid	Pectins	Total sugars	Reducing sugars	Moisture
Wood apple	3·72	3·95	6·86	4·51	71·8
Guava	0·32	1·44	16·62	6·12	78·8
Karaunda	3·1	1·23	2·23	1·22	89·0
Roselle—					
A. Calyx	3·74	3·19	3·34	2·41	88·2
B. Entire fruit	2·05	2·48	3·1	1·22	82·66
C. Fruit	1·03	1·02	2·28	1·05	76·4
Orange	1·17	1·2	11·53	5·4	88·6
Lemon	7·47	2·76	0·84	..	85·08
Jujube (*ber*)	1·12	1·45	15·78	7·23	80·01
Banana	0·3	1·19	19·98	6·78	73·6
Bel	0·51	2·03	13·04	5·26	68·8
Cape gooseberry	2·19	0·75	15·56	6·78	83·8

TABLE III

Effect of sugar

[On wood apple juice containing 0·238 gm. acid (citric) and 0·07 gm. pectin (calcium pectate)]

Sugar added in gm.	Yield of jelly (gm.)	Jelly strength	Sineresis temperature (°C.)	Percentage of acid	Percentage of pectin	Percentage of sugar
10	16·5	120	64·5	1·44	0·42	60·5
12·5	20·5	84	61	1·11	0·34	61
15	24	75	56	0·95	0·29	62·5
20	31·6	70	50	0·75	0·22	63·2
30	47	27	41·5	0·50	0·15	63·83
40	63·8	13	34	0·37	0·11	64·2
50	77·2	9	Room temp.	0·31	0·09	64·7

TABLE IV

Effect of sugar

[On guava juice containing 0·418 gm. acid (citric) and 0.076 gm. pectin (calcium pectate)]

Sugar added in gm.	Yield of jelly (gm.)	Jelly strength	Sineresis temp. (°C.)	Acid (per cent)	Pectin (per cent)	Sugar (per cent)
10	16·5	180	66	2·53	0·46	60·5
15	24	85	60·5	1·74	0·32	62·5
20	32	74	52·5	1·3	0·24	62·5
30	47	62	47	0·89	0·16	63·8
40	62·5	12	32	0·67	0·12	64
45	70	9	Room temp.	0·59	0·11	64·3
50	77·5	No gel	..	0·45	0·09	64·5

TABLE V

Effect of sugar

On roselle juice containing 0·235 gm. acid (citric) and 0·12 gm. pectin (calcium pectate)]

Sugar added in gm.	Yield of jelly (gm.)	Jelly strength	Sineresis temperature (°C.)	Acid (per cent)	Pectin (per cent)	Sugar (per cent)
10	16·5	210	72	1·43	0·73	60·5
15	24·5	105	65	0·96	0·49	61·2
20	32·2	83	62	0·73	0·37	62·1
25	39·7	32	43	0·59	0·30	63·0
30	47·4	15	36·5	0·49	0·26	63·4
35	55	9	Room temp.	0·42	0·22	63·7
40	62·5	No gel	..	0·37	0·19	64·1

TABLE VI

Effect of acids

(Figures indicate the strength of the wood apple jellies obtained, containing 59·5 per cent sugar and 0·357 per cent pectin)

Normal solution in c.c. added	Citric acid	Tartaric acid	Hydrochloric acid
0·5	58	61	76
1	65	70	70
2	70	76	42
3	73	84	10
5	80	75	No gel
7	72	67	..
10	58	45	..

TABLE VII

Effect of acids

(Figures indicate the strength of guava jellies obtained, containing 59.5 per cent sugar and 0·286 per cent pectin)

Normal solution in c.c. added	Citric acid	Tartaric acid	Hydrochloric acid
0.5	No gel	No gel	67
1	,,	,,	53
2	,,	,,	32
3	,,	,,	11
5	,,	12	9
7	14	48	No gel
10	42	55	..
15	56	72	..
20	70	64	..
25	54	42	..

TABLE VIII

Effect of acids

[Showing the sugar-holding capacity of *Karounda* pectin (0·095 gm.) with increased acidity]

Acid added in gm.	Sugar required (gm.)	Yield of jelly (gm.)	Percentage of acid	Percentage of sugar
None	40	62·7	0·51	63·7
0·25	42	65·4	0·87	64·2
0·5	43	66·1	1·24	65·05
0·75	44	66·8	1·6	65·8
1·0	44	66·2	1·99	66·4

TABLE IX

Effect of acids

[Showing the sugar-holding capacity of guava pectin (0·134 gm.) with increased acidity]

Acid added in gm.	Sugar required (gm.)	Yield of jelly (gm.)	Percentage of acid	Percentage of sugar
0·25	30	46·5	0·63	64·5
0·5	32	49·3	1·1	64·9
0·75	35	54·2	1·46	64·6
1·0	37	56·8	1·8	65·1
1·5	38	58·1	2·65	65·4

TABLE X

Effect of pectin

(Showing decrease in the amount of sugar with increase of wood apple pectin solution)

Pectin solution added in c.c.	Total amount of pectin (gm.)	Sugar required (gm.)	Percentage of pectin	Percentage of sugar
None	0·07	30	0·16	66·6
5	0·23	27·5	0·51	61
10	0·39	25·5	0·86	57
15	0·55	24	1·22	53·3
20	0·71	23	1·57	51·1
25	0·87	22·5	1·93	50
30	1·03	22	2·28	48·8

TABLE XI

Effect of pectin

(Showing decrease in the amount of sugar with increase of pectin from roselle calyx)

Pectin solution added (c.c.)	Total pectin (gm.)	Sugar required (gm.)	Percentage of pectin	Percentage of sugar
None	0·14	40	0·24	67·8
5	0·38	36·5	0·64	61·8
10	0·62	33·5	1·05	56·7
15	0·86	31·5	1·45	53·4
20	1·10	30	1·86	50·1
25	1·34	29	2·27	49·1
30	1·58	28·5	2·67	48.3

NOTE

BIOLOGICAL CONTROL OF LANTANA

LANTANA, originally considered as an ornamental plant, has assumed the status of a noxious weed in several parts of the world, including India. The Forest Departments in India are generally agreed that it is a weed with no prospects of economical utilization and should, if possible, be replaced by tree crops.

Several attempts have been made to exterminate the weed by biological methods of control in various parts of the world. The bug *Teleonemia lantanæ* Distant (=*T. scrupulosa* Stal) has proved most promising from this viewpoint. This bug was introduced into Hawaii, Australia, Fiji, etc. for the control of lantana and has proved most useful in Australia. It is probable that in one or more of the climatic regions in south India the bug will flourish, but it perhaps cannot be relied upon to produce wholesale destruction of the weed throughout India. In this connection, it should be remembered that the family *Verbanaceæ*, which includes lantana, also includes teak and several plants, e.g. *Verbena*, *Vitex negunda*, *Aloysia citrioldora*, species of *Clerodendron*, which are ornamental plants in India. This bug has been found to attack *Callirhœ involucrata* and an undetermined species of Labiatæ in some parts of the world.

Thus the bug has potentialities for evil also. One has to be sure that by introducing an undesirable lantana insect a problem more difficult to solve than the extermination of lantana itself is not created. One should not go by what plants are known to be attacked by this insect in other parts of the world, but should determine for oneself what it would attack in India. Almost every important country, which introduced this bug, did so after exhaustive tests on economic plants under quarantine conditions. The Forest Entomologist, Forest Research Institute, Dehra Dun, is investigating the capabilities of the bug. In the meantime, the Government of India have notified Provincial Governments not to release the insect *Teleonemia lantanae* for the control of lantana weed till the characteristics of the insect have been more fully investigated, and not without the fullest consultation with the expert authorities, especially the Forest Entomologist, Forest Research Institute and College, Dehra Dun, and the Imperial Entomologist, Imperial Agricultural Research Institute, New Delhi.

REVIEW

Deltaic Formation. By C. Strickland (Longmans, Green & Co. Ltd., 1940. Pp. 158 : Rs. 5)

PROFESSOR Debenham writes in the foreword : ' It is unusual but not unique to find an expert in one branch of science applying himself to problems in another branch removed from his own. The result often is that new light is thrown upon the subject, viewed as it must be from an aspect totally different to that used by its own specialists. ....Naturally his terminology and his metaphor are unusual, but that should not prevent the reader from understanding the author's outlook. ' And Dr Cyril Fox in his introduction joins issue with the author regarding the correctness of the term Ganges Delta and states : ' The Ganges Delta on the other head represents so much country so to speak abandoned by this great river as it has adopted new channels from its original course down the Hughli to its present junction with the new Brahmaputra. The latter river also does not give off any actual distributaries.' But concludes,' However, while I may argue about the correctness of the term Ganges Delta there can be no doubt of its mode of formation which Dr Strickland describes so fully .'

The book is based on personal knowledge acquired in course of tours and travels in connection with the author's study of the conditions and circumstances under which malaria develops and thrives. As he tells us in the preface the book is ' a contribution to physiography not only for the use of those engaged in the prevention of malaria in the tropics but also for that of other administrators such as those who have to deal with irrigation, agricultural science, forests, land-settlement town-planning, water supplies, port and river conservancy, rural sanitation, road and railway construction, bridge building and in fact those who should base their schemes on a succinct knowledge of what Nature is doing under their very noses. ' The reviewer fully subscribes to the view that correct and more complete knowledge of this region will be of immense public benefit. Schemes of public utility, if they are to be sound and fruitful, have to be based on the results of scientific enquiry. It cannot be denied, however, that the problems confronting such enquiries require the attention of experts in several branches of science and perhaps the very best of them. River physics is a very specialized branch of study, so also is the problem of soil genesis. It is on the facts ascertained by physicists, river engineers, geologists and soil scientists and, though it may not be so apparent, by botanists and zoologists that the geographer has to base his thesis and his correlated general picture of the geographical features of a region.

The quotations given above explain the scope and interest of this book. The detailed scientific study of the Lower Bengal has, however, scarcely begun, although in addition to its practical interest a full knowledge of its past and present conditions is of general scientific interest.

Dr Strickland has undoubtedly earned the thanks of all interested in the Ganges delta in undertaking the task of writing this interesting book on the manner of formation of this region and thereby stimulating thought and attention to a scientific study of this region. The state and the public have definitely a duty to perform in fostering such studies and should not leave them to the offchance of an enthusiast undertaking this work who, however qualified he may be, can scarcely encompass the whole field of enquiry or gather the basic data unaided by a body of experts in allied fields and within a reasonable interval of time.

The book is divided into 27 chapters dealing with various subjects including the following :

Scope of the enquiry ; what is meant by a ' Delta ' ; the birth and infancy of the land ; a digression on alluvial fans ; floods ; the flood-plain ; land and water profiles in relation to the tides ; ground and surface water ; seepage ; varying character of the sedimentation and stratification ; meandering ; *bhils* and *jheels* ; salinity of the rivers of the Delta ; and the influence of diastrophism of the Earth's crust on the hydrographic processes.

His main thesis appears to be as follows : Only hydrographic processes as opposed to diastrophic processes are responsible for the formation of the Ganges delta and the *bhils*, lakes, alluvial fans and others which occur in this region. Hydrographic processes are of course major contributory factors to the formation of deltaic tracts but he denies, particularly with regard to Bengal Delta, the influence of diastrophic changes suggested by some geologists and geodesists. In order to settle this controversial question it seems necessary to make deep borings similar to that put down at Fort William in Calcutta in 1919 in various regions with a view to examining in detail the nature of the deposits. This work, however, involves considerable expense. The available data are not sufficient to fully support the author's contentions mentioned above and the following observations illustrate the difficulties which the reviewer has felt in accepting them.

1. It is rather doubtful that the ' swatch-of-no-ground ', a deep chasm of the size and shape found at the head of the Bay of Bengal would be formed merely by hydrographic processes. The author does not attempt to indicate how the magnitude of the forces which are involved can be produced by hydrographic factors of the type consiered by the author. To the reviewer diastrophic changes appear to offer a more correct explanation. (2) The author's characterization of a ' delta ' as ' depositing ' and of ' paradelta ' as ' eroding ' does not seem to have much significance. Is it not an usual occurrence that rivers erode one bank and lay deposits on the other or swing from position to position or change their course as a result of erosion and deposition ? What part do the variations in the rainfall and the run-off to rivers play in affecting the course of rivers ? Or in other words, does the ' degrading ' and ' aggrading ' action of a river work so smoothly through geological periods of time ? (3) According to the author the Madhupur Jungle tract, the Barind of North Bengal, the red bank of Comilla, etc. are nothing but terraces of old deposits of glacial origin that have so far escaped degradation by the erosive action of the Ganges, its tributaries and distributaries. Ordinarily the erosive action of rivers is weakest towards the sea face and greatest upwards. The relative sizes of the residual masses of land comprising these

tracts are, however, contrary to what is expected from the above consideration. More concrete data regarding the courses of the rivers at various times, and the geology of the land masses are required for critical correlation. (4) The processes discussed by the author of the formation of *bhils* in the Lower Bengal appears to be suggestive. But ephemeral changes to which the author refers in the last chapter and which mask the effects of secular variations might be as well caused by very weak crustal movements.

In connection with a soil survey in the coastal regions of the Bakharganj district, the reviewer had occasion to visit some of these tracts, which comprise recent *char* land and lands formed about 150-200 years back. They lie somewhat inland about 40 miles from the sea and are formed by the deposition of the large volume of suspended matter carried by the rivers. No evidence has been obtained of the type of aggrading or degrading action mentioned by the author. By means of profile examination together with physical and chemical analyses it has been possible to obtain a general picture of the formation of these tracts. The deposits consist of layers about 2-3 mm. thick of clay and silt separated by almost a unigranular deposit of fine sand or material of coarser texture. These 'horizons' in the soil profile have been termed 'deposition horizons' which are shown very clearly by recently formed *chars*. From the number of such layers it seems possible to count the number of tidal flows and ebbs which produce a given depth of the soil. Although unusual floods leave their mark on the whole profile, the process of formation of the soil suggested by these 'deposition horizons' is very uniform over this tract. In older *char* lands the top deposits are disturbed through cultivation and other influences. These interferences during the raising of the land tend to form a hard subsoil layer by the accumulation of finer particles, e.g., clay and silt. This is probably the origin of 'clay pans' in the coastal areas, which have been observed so far in the older *chars*.

As already mentioned the study of the formation of this deltaic tract should be placed on a more scientific basis. For this it seems that deep borings and analyses of the deposits at different depths by mineralogical, physical and chemical methods would throw considerable light on the nature of the deposits, the manner of transport and the mode of formation of the tract.

The book is generally free from printing and other errors. A few which caught the reviewer's eyes are mentioned below :

(1) 'Madaripur Jungle' in the map on page 9 should be 'Madhupur Jungle'.

(2) There is no reference to 'swatch-of-no-ground' on page 9, although the index mentions this ; the reference is perhaps to page 12, of which the index, however, makes no mention.

(3) 'Indeed it has been elsewhere stated that his (Rennel's) survey .....methods' (page 118). No such reference is to be found in the book. [J. N. M.]

PLANT QUARANTINE NOTIFICATIONS

THE Imperial Agricultural Bureaux have just issued the 10-year Subject and Author Index to *Horticultural Abstracts* 1931-40. Price about 25*s*. (No free issue.)

All orders should be sent direct to The Imperial Agricultural Bureaux, Central Sales Branch, Agricultural Research Building, Penglais, Aberystwyth, Wales

INDIA

Form of special permit authorizing importation of insects

[Prescribed by the Central Government under para. 2(*a*) of the Notification* No. F.-193/40-A, dated 3 February 1941]

1. Name, designation and full address of the importer . . . . . .
2. Name of the insect species to be imported . . . . . .
3. Stage or states of the insect to be imported . . . . . .
4. Country from which importation is sought . . . . . .
5. Whether importation is intended by sea, land or air . . . . . .
6. Whether in its original home it is a weed pest, a parasite or a predator. . . . . .
   (*i*) Name (names) of the weed (weeds) on which it is a pest in the country of origin . . . . . .
   (*ii*) Name (names) of the pest (pests) on which it is a parasite or predator in the country of origin . . . . . .
7. Name, designation and address of the exporter . . . . . .
8. Quantity indented for . . . . . .
9. Purpose of importation . . . . . .

I authorize the importation. This permit will be valid up to.....

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(Signature and designation of the certifying authority)

Date.....

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*Published in this Journal, Vol. 11, Part II, page 322

Notification No. F. 193/40-A. (c), *dated 12 August 1941 of the Government of India in the Department of Education, Health and Lands*

IN exercise of the powers conferred by sub-section (1) of section 3 of the Destructive Insects and Pests Act, 1914 (II of 1914), the Central Government is pleased to direct that the following amendment shall be made in the Order published with the notification of the Government of India in the Department of Education, Health and Lands, No. F.-193/40-A., dated the 3rd February 1941, namely :—

In clause (*b*) of paragraph 3 of the said Order, after the word ' Orissa ' the words ' Jammu and Kashmir ' shall be inserted.

G. S. BOZMAN

Joint Secretary to the Government of India

Notification No. F. 15-11/41-A., dated 1 September 1941 of the Government of India in the Department of Education, Health and Lands

IN exercise of the powers conferred by sub-section (1) of section 3 of the Destructive Insects and Pests Act, 1914 (II of 1914), the Central Government is pleased to direct that the following further amendment shall be made in the Order published with the notification of the Government of India in the Department of Education, Health and Lands, No. F. 320-35-A., dated the 20th July 1936, namely :—

In sub-paragraph (2) of paragraph 9 of the said Order for the words and brackets ' (*Ceratostomela paradoxa* or *Thielaviopsis paradoxa*) ' the words and brackets ' *Ceratostomella paradoxa* (Thielaviopsis paradoxa) ' shall be substituted.

G. S. BOZMAN

Joint Secretary to the Government of India.

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Page 543 (appendix), column 3, line 10, *for* ' 16,500·0 ' *read* ' 116,500·0 '

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(December 1941)

The Editorial Committee of the Imperial Council of Agricultural Research, India, takes no responsibility for the opinions expressed in this Journal.